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(54) Title: GENES OF CORYNEBACTERIUM

(57) Abstract: Isolated nucleic acid molecules, designated MP nucleic acid molecules, which encode novel MP proteins from *Corynebacterium glutamicum* are described. The invention also provides antisense nucleic acid molecules, recombinant expression vectors containing MP nucleic acid molecules, and host cells into which the expression vectors have been introduced. The invention still further provides isolated MP proteins, mutated MP proteins, fusion proteins, antigenic peptides and methods for the improvement of production of a desired compound from *C. glutamicum* based on genetic engineering of MP genes in this organism.

GENES OF CORYNEBACTERIUM

Isolated nucleic acid molecules, designated MP nucleic acid
5 molecules, which encode novel MP proteins from *Corynebacterium glutamicum* are described. The invention also provides antisense nucleic acid molecules, recombinant expression vectors containing MP nucleic acid molecules, and host cells into which the expression vectors have been introduced. The invention still
10 further provides isolated MP proteins, mutated MP proteins, fusion proteins, antigenic peptides and methods for the improvement of production of a desired compound from *C. glutamicum* based on genetic engineering of MP genes in this organism.

15 Certain products and by-products of naturally-occurring metabolic processes in cells have utility in a wide array of industries, including the food, feed, cosmetics, and pharmaceutical industries. These molecules, collectively termed 'fine
20 chemicals', include organic acids, both proteinogenic and non-proteinogenic amino acids, nucleotides and nucleosides, lipids and fatty acids, diols, carbohydrates, aromatic compounds, vitamins and cofactors, and enzymes. Their production is most conveniently performed through large-scale culture of bacteria
25 developed to produce and secrete large quantities of a particular desired molecule. One particularly useful organism for this purpose is *Corynebacterium glutamicum*, a gram positive, nonpathogenic bacterium. Through strain selection, a number of mutant strains have been developed which produce an array of
30 desirable compounds. However, selection of strains improved for the production of a particular molecule is a time-consuming and difficult process.

The invention provides novel bacterial nucleic acid molecules
35 which have a variety of uses. These uses include the identification of microorganisms which can be used to produce fine chemicals, the modulation of fine chemical production in *C. glutamicum* or related bacteria, the typing or identification of *C. glutamicum* or related bacteria, as reference points
40 for mapping the *C. glutamicum* genome, and as markers for transformation. These novel nucleic acid molecules encode proteins, referred to herein as metabolic pathway (MP) proteins.

C. glutamicum is a gram positive, aerobic bacterium which is
45 commonly used in industry for the large-scale production of a variety of fine chemicals, and also for the degradation of hydrocarbons (such as in petroleum spills) and for the oxidation

of terpenoids. The MP nucleic acid molecules of the invention, therefore, can be used to identify microorganisms which can be used to produce fine chemicals, e.g., by fermentation processes. Modulation of the expression of the MP nucleic acids of the invention, or modification of the sequence of the MP nucleic acid molecules of the invention, can be used to modulate the production of one or more fine chemicals from a microorganism (e.g., to improve the yield or production of one or more fine chemicals from a *Corynebacterium* or *Brevibacterium* species).

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The MP nucleic acids of the invention may also be used to identify an organism as being *Corynebacterium glutamicum* or a close relative thereof, or to identify the presence of *C. glutamicum* or a relative thereof in a mixed population of microorganisms. The invention provides the nucleic acid sequences of a number of *C. glutamicum* genes; by probing the extracted genomic DNA of a culture of a unique or mixed population of microorganisms under stringent conditions with a probe spanning a region of a *C. glutamicum* gene which is unique to this organism, one can ascertain whether this organism is present. Although *Corynebacterium glutamicum* itself is nonpathogenic, it is related to species pathogenic in humans, such as *Corynebacterium diphtheriae* (the causative agent of diphtheria); the detection of such organisms is of significant clinical relevance.

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The MP nucleic acid molecules of the invention may also serve as reference points for mapping of the *C. glutamicum* genome, or of genomes of related organisms. Similarly, these molecules, or variants or portions thereof, may serve as markers for genetically engineered *Corynebacterium* or *Brevibacterium* species..

The MP proteins encoded by the novel nucleic acid molecules of the invention are capable of, for example, performing an enzymatic step involved in the metabolism of certain fine chemicals, including amino acids, vitamins, cofactors, nutraceuticals, nucleotides, nucleosides, and trehalose. Given the availability of cloning vectors for use in *Corynebacterium glutamicum*, such as those disclosed in Sinskey et al., U.S. Patent No. 4,649,119, and techniques for genetic manipulation of *C. glutamicum* and the related *Brevibacterium* species (e.g., *lactofermentum*) (Yoshihama et al, *J. Bacteriol.* 162: 591-597 (1985); Katsumata et al., *J. Bacteriol.* 159: 306-311 (1984); and Santamaria et al., *J. Gen. Microbiol.* 130: 2237-2246 (1984)), the nucleic acid molecules of the invention may be utilized in the genetic engineering of this organism to make it a better or more efficient producer of one or more fine chemicals.

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This improved production or efficiency of production of a fine chemical may be due to a direct effect of manipulation of a gene of the invention, or it may be due to an indirect effect of such manipulation. Specifically, alterations in *C. glutamicum* metabolic pathways for amino acids, vitamins, cofactors, nucleotides, and trehalose may have a direct impact on the overall production of one or more of these desired compounds from this organism. For example, optimizing the activity of a trehalose or a lysine or a methionine biosynthetic pathway protein or decreasing the activity of a trehalose or a lysine or methionine degradative pathway protein may result in an increase in the yield or efficiency of production of trehalose or lysine or methionine from such an engineered organism. Alterations in the proteins involved in these metabolic pathways may also have an indirect impact on the production or efficiency of production of a desired fine chemical. For example, a reaction which is in competition for an intermediate necessary for the production of a desired molecule may be eliminated, or a pathway necessary for the production of a particular intermediate for a desired compound may be optimized. Further, modulations in the biosynthesis or degradation of, for example, an amino acid, a vitamin, or a nucleotide may increase the overall ability of the microorganism to rapidly grow and divide, thus increasing the number and/or production capacities of the microorganism in culture and thereby increasing the possible yield of the desired fine chemical.

The nucleic acid and protein molecules of the invention may be utilized to directly improve the production or efficiency of production of one or more desired fine chemicals from *Corynebacterium glutamicum*. Using recombinant genetic techniques well known in the art, one or more of the biosynthetic or degradative enzymes of the invention for amino acids, vitamins, cofactors, nutraceuticals, nucleotides, nucleosides, or trehalose may be manipulated such that its function is modulated. For example, a biosynthetic enzyme may be improved in efficiency, or its allosteric control region destroyed such that feedback inhibition of production of the compound is prevented. Similarly, a degradative enzyme may be deleted or modified by substitution, deletion, or addition such that its degradative activity is lessened for the desired compound without impairing the viability of the cell. In each case, the overall yield or rate of production of the desired fine chemical may be increased.

It is also possible that such alterations in the protein and nucleotide molecules of the invention may improve the production of other fine chemicals besides the amino acids, vitamins,

cofactors, nutraceuticals, nucleotides, nucleosides, and trehalose through indirect mechanisms. Metabolism of any one compound is necessarily intertwined with other biosynthetic and degradative pathways within the cell, and necessary cofactors, intermediates, or substrates in one pathway are likely supplied or limited by another such pathway. Therefore, by modulating the activity of one or more of the proteins of the invention, the production or efficiency of activity of another fine chemical biosynthetic or degradative pathway may be impacted. For example, amino acids serve as the structural units of all proteins, yet may be present intracellularly in levels which are limiting for protein synthesis; therefore, by increasing the efficiency of production or the yields of one or more amino acids within the cell, proteins, such as biosynthetic or degradative proteins, may be more readily synthesized. Likewise, an alteration in a metabolic pathway enzyme such that a particular side reaction becomes more or less favored may result in the over- or under-production of one or more compounds which are utilized as intermediates or substrates for the production of a desired fine chemical.

This invention provides novel nucleic acid molecules which encode proteins, referred to herein as metabolic pathway proteins (MP), which are capable of, for example, performing an enzymatic step involved in the metabolism of molecules important for the normal functioning of cells, such as amino acids, vitamins, cofactors, nucleotides and nucleosides, or trehalose. Nucleic acid molecules encoding an MP protein are referred to herein as MP nucleic acid molecules. In a preferred embodiment, the MP protein performs an enzymatic step related to the metabolism of one or more of the following: amino acids, vitamins, cofactors, nutraceuticals, nucleotides, nucleosides, and trehalose. Examples of such proteins include those encoded by the genes set forth in Table 1.

Table 1: Genes in the Application

40	Nucleic Acid SEQ ID NO	Amino Acid SEQ ID NO	Gene name (identifier)	Function
	1	2	metH	5-Methyltetrahydrofolate-homocysteine methyltransferase (EC 2.1.1.13)
	3	4	treS	Trehalose Synthase

Accordingly, one aspect of the invention pertains to isolated nucleic acid molecules (e.g., cDNAs, DNAs, or RNAs) comprising a nucleotide sequence encoding an MP protein or biologically active portions thereof, as well as nucleic acid fragments suitable as

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primers or hybridization probes for the detection or amplification of MP-encoding nucleic acid (e.g., DNA or mRNA). In particularly preferred embodiments, the isolated nucleic acid molecule comprises one of the nucleotide sequences set forth as the odd-numbered SEQ ID NOs in the Sequence Listing (SEQ ID NO:1, SEQ ID NO:3), or the coding region or a complement thereof of one of these nucleotide sequences. In other particularly preferred embodiments, the isolated nucleic acid molecule of the invention comprises a nucleotide sequence which hybridizes to or is at least about 63%, preferably at least about 71%, more preferably at least about 75%, 80% or 90%, and even more preferably at least about 95%, 96%, 97%, 98%, 99% or more homologous to a nucleotide sequence which encodes a protein sequence set forth as an even-numbered SEQ ID NO in the Sequence Listing (SEQ ID NO:2, SEQ ID NO:4), or a portion thereof. In other preferred embodiments, the isolated nucleic acid molecule encodes one of the amino acid sequences set forth as an even-numbered SEQ ID NO in the Sequence Listing (SEQ ID NO:2, SEQ ID NO:4). The preferred MP proteins of the present invention also preferably possess at least one of the MP activities described herein.

In another embodiment, the isolated nucleic acid molecule encodes a protein or portion thereof wherein the protein or portion thereof includes an amino acid sequence which is sufficiently homologous to an amino acid sequence of the invention (e.g., a sequence having an even-numbered SEQ ID NO: in the Sequence Listing), e.g., sufficiently homologous to an amino acid sequence of the invention such that the protein or portion thereof maintains an MP activity. Preferably, the protein or portion thereof encoded by the nucleic acid molecule maintains the ability to perform an enzymatic reaction in a amino acid, vitamin, cofactor, nutraceutical, nucleotide, nucleoside, or trehalose metabolic pathway. In one embodiment, the protein encoded by the nucleic acid molecule is at least about 63%, preferably at least about 71%, and more preferably at least about 75%, 80%, or 90% and most preferably at least about 95%, 96%, 97%, 98%, or 99% or more homologous to an amino acid sequence of the invention (e.g., an entire amino acid sequence selected from those having an even-numbered SEQ ID NO in the Sequence Listing).

In another preferred embodiment, the protein is a full length C. glutamicum protein which is substantially homologous to an entire amino acid sequence of the invention (encoded by an open reading frame shown in the corresponding odd-numbered SEQ ID NOs in the Sequence Listing (SEQ ID NO:2, SEQ ID NO:4)).

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In another preferred embodiment, the isolated nucleic acid molecule is derived from *C. glutamicum* and encodes a protein (e.g., an MP fusion protein) which includes a biologically active domain which is at least about 50% or more homologous to one of the amino acid sequences of the invention (e.g., a sequence of one of the even-numbered SEQ ID NOs in the Sequence Listing) and is able to catalyze a reaction in a metabolic pathway for an amino acid, vitamin, cofactor, nutraceutical, nucleotide, nucleoside, or trehalose, or one or more of the activities set forth in Table 1, and which also includes heterologous nucleic acid sequences encoding a heterologous polypeptide or regulatory regions.

In another embodiment, the isolated nucleic acid molecule is at least 15 nucleotides in length and hybridizes under stringent conditions to a nucleic acid molecule comprising a nucleotide sequence of the invention (e.g., a sequence of an odd-numbered SEQ ID NO in the Sequence Listing). Preferably, the isolated nucleic acid molecule corresponds to a naturally-occurring nucleic acid molecule. More preferably, the isolated nucleic acid encodes a naturally-occurring *C. glutamicum* MP protein, or a biologically active portion thereof.

Another aspect of the invention pertains to vectors, e.g., recombinant expression vectors, containing the nucleic acid molecules of the invention, and host cells into which such vectors have been introduced. In one embodiment, such a host cell is used to produce an MP protein by culturing the host cell in a suitable medium. The MP protein can be then isolated from the medium or the host cell.

Yet another aspect of the invention pertains to a genetically altered microorganism in which an MP gene has been introduced or altered. In one embodiment, the genome of the microorganism has been altered by introduction of a nucleic acid molecule of the invention encoding wild-type or mutated MP sequence as a transgene. In another embodiment, an endogenous MP gene within the genome of the microorganism has been altered, e.g., functionally disrupted, by homologous recombination with an altered MP gene. In another embodiment, an endogenous or introduced MP gene in a microorganism has been altered by one or more point mutations, deletions, or inversions, but still encodes a functional MP protein. In still another embodiment, one or more of the regulatory regions (e.g., a promoter, repressor, or inducer) of an MP gene in a microorganism has been altered (e.g., by deletion, truncation, inversion, or point mutation) such that the expression of the MP gene is modulated. In a preferred

embodiment, the microorganism belongs to the genus *Corynebacterium* or *Brevibacterium*, with *Corynebacterium glutamicum* being particularly preferred. In a preferred embodiment, the microorganism is also utilized for the production of a desired compound, such as trehalose or an amino acid, with lysine and methionine being particularly preferred.

In another aspect, the invention provides a method of identifying the presence or activity of *Corynebacterium diphtheriae* in a subject. This method includes detection of one or more of the nucleic acid or amino acid sequences of the invention (e.g., the sequences set forth in the Sequence Listing as SEQ ID NOs 1 through 4) in a subject, thereby detecting the presence or activity of *Corynebacterium diphtheriae* in the subject.

Still another aspect of the invention pertains to an isolated MP protein or a portion, e.g., a biologically active portion, thereof. In a preferred embodiment, the isolated MP protein or portion thereof can catalyze an enzymatic reaction involved in one or more pathways for the metabolism of an amino acid, a vitamin, a cofactor, a nutraceutical, a nucleotide, a nucleoside, or trehalose. In another preferred embodiment, the isolated MP protein or portion thereof is sufficiently homologous to an amino acid sequence of the invention (e.g., a sequence of an even-numbered SEQ ID NO: in the Sequence Listing) such that the protein or portion thereof maintains the ability to catalyze an enzymatic reaction involved in one or more pathways for the metabolism of an amino acid, a vitamin, a cofactor, a nutraceutical, a nucleotide, a nucleoside, or trehalose.

The invention also provides an isolated preparation of an MP protein. In preferred embodiments, the MP protein comprises an amino acid sequence of the invention (e.g., a sequence of an even-numbered SEQ ID NO: of the Sequence Listing). In another preferred embodiment, the invention pertains to an isolated full length protein which is substantially homologous to an entire amino acid sequence of the invention (e.g., a sequence of an even-numbered SEQ ID NO: of the Sequence Listing) (encoded by an open reading frame set forth in a corresponding odd-numbered SEQ ID NO: of the Sequence Listing). In yet another embodiment, the protein is at least about 63%, preferably at least about 71%, and more preferably at least about 75%, 80%, or 90%, and most preferably at least about 95%, 96%, 97%, 98%, or 99% or more homologous to an entire amino acid sequence of the invention (e.g., a sequence of an even-numbered SEQ ID NO: of the Sequence Listing). In other embodiments, the isolated MP protein comprises an amino acid sequence which is at least about 63% or more

homologous to one of the amino acid sequences of the invention (e.g., a sequence of an even-numbered SEQ ID NO: of the Sequence Listing) and is able to catalyze an enzymatic reaction in an amino acid, vitamin, cofactor, nutraceutical, nucleotide, nucleoside, or trehalose metabolic pathway, or has one or more of the activities set forth in Table 1.

Alternatively, the isolated MP protein can comprise an amino acid sequence which is encoded by a nucleotide sequence which hybridizes, e.g., hybridizes under stringent conditions, or is at least about 63%, preferably at least about 71%, more preferably at least about 70%, 80%, or 90%, and even more preferably at least about 95%, 96%, 97%, 98%, or 99% or more homologous to a nucleotide sequence encoding a protein of one of the even-numbered SEQ ID NOs set forth in the Sequence Listing. It is also preferred that the preferred forms of MP proteins also have one or more of the MP bioactivities described herein.

The MP polypeptide, or a biologically active portion thereof, can be operatively linked to a non-MP polypeptide to form a fusion protein. In preferred embodiments, this fusion protein has an activity which differs from that of the MP protein alone. In other preferred embodiments, this fusion protein, when introduced into a *C. glutamicum* pathway for the metabolism of an amino acid, vitamin, cofactor, nutraceutical, results in increased yields and/or efficiency of production of a desired fine chemical from *C. glutamicum*. In particularly preferred embodiments, integration of this fusion protein into an amino acid, vitamin, cofactor, nutraceutical, nucleotide, nucleoside, or trehalose metabolic pathway of a host cell modulates production of a desired compound from the cell.

In another aspect, the invention provides methods for screening molecules which modulate the activity of an MP protein, either by interacting with the protein itself or a substrate or binding partner of the MP protein, or by modulating the transcription or translation of an MP nucleic acid molecule of the invention.

Another aspect of the invention pertains to a method for producing a fine chemical. This method involves the culturing of a cell containing a vector directing the expression of an MP nucleic acid molecule of the invention, such that a fine chemical is produced. In a preferred embodiment, this method further includes the step of obtaining a cell containing such a vector, in which a cell is transfected with a vector directing the expression of an MP nucleic acid. In another preferred embodiment, this method further includes the step of recovering

the fine chemical from the culture. In a particularly preferred embodiment, the cell is from the genus *Corynebacterium* or *Brevibacterium*, or is selected from those strains set forth in Table 2.

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Table 2: *Corynebacterium* and *Brevibacterium* strains which may be used in the practice of the invention

	Genus	Species	ATCC	FERM	NRRL	CECT	NCIMB	CBS	NCTC	DSMZ
10	<i>Brevibacterium</i>	<i>Ammoniagenes</i>	21054							
	<i>Brevibacterium</i>	<i>Ammoniagenes</i>	19350							
	<i>Brevibacterium</i>	<i>Ammoniagenes</i>	19351							
	<i>Brevibacterium</i>	<i>Ammoniagenes</i>	19352							
	<i>Brevibacterium</i>	<i>Ammoniagenes</i>	19353							
	<i>Brevibacterium</i>	<i>Ammoniagenes</i>	19354							
15	<i>Brevibacterium</i>	<i>Ammoniagenes</i>	19355							
	<i>Brevibacterium</i>	<i>Ammoniagenes</i>	19356							
	<i>Brevibacterium</i>	<i>Ammoniagenes</i>	21055							
	<i>Brevibacterium</i>	<i>Ammoniagenes</i>	21077							
	<i>Brevibacterium</i>	<i>Ammoniagenes</i>	21553							
	<i>Brevibacterium</i>	<i>Ammoniagenes</i>	21580							
20	<i>Brevibacterium</i>	<i>Ammoniagenes</i>	39101							
	<i>Brevibacterium</i>	<i>Butanicum</i>	21196							
	<i>Brevibacterium</i>	<i>Divaricatum</i>	21792	P928						
	<i>Brevibacterium</i>	<i>Flavum</i>	21474							
	<i>Brevibacterium</i>	<i>Flavum</i>	21129							
	<i>Brevibacterium</i>	<i>Flavum</i>	21518							
25	<i>Brevibacterium</i>	<i>Flavum</i>			B11474					
	<i>Brevibacterium</i>	<i>Flavum</i>			B11472					
	<i>Brevibacterium</i>	<i>Flavum</i>	21127							
	<i>Brevibacterium</i>	<i>Flavum</i>	21128							
	<i>Brevibacterium</i>	<i>Flavum</i>	21427							
	<i>Brevibacterium</i>	<i>Flavum</i>	21475							
30	<i>Brevibacterium</i>	<i>Flavum</i>	21517							
	<i>Brevibacterium</i>	<i>Flavum</i>	21528							
	<i>Brevibacterium</i>	<i>Flavum</i>	21529							
	<i>Brevibacterium</i>	<i>Flavum</i>			B11477					
	<i>Brevibacterium</i>	<i>Flavum</i>			B11478					
	<i>Brevibacterium</i>	<i>Flavum</i>	21127							
35	<i>Brevibacterium</i>	<i>Flavum</i>			B11474					
	<i>Brevibacterium</i>	<i>Healii</i>	15527							
	<i>Brevibacterium</i>	<i>Ketoglutamicum</i>	21004							
	<i>Brevibacterium</i>	<i>Ketoglutamicum</i>	21089							
	<i>Brevibacterium</i>	<i>Ketosoreductum</i>	21914							
	<i>Brevibacterium</i>	<i>Lactofermentum</i>				70				
40	<i>Brevibacterium</i>	<i>Lactofermentum</i>				74				
	<i>Brevibacterium</i>	<i>Lactofermentum</i>				77				
	<i>Brevibacterium</i>	<i>Lactofermentum</i>	21798							
	<i>Brevibacterium</i>	<i>Lactofermentum</i>	21799							
	<i>Brevibacterium</i>	<i>Lactofermentum</i>	21800							
	<i>Brevibacterium</i>	<i>Lactofermentum</i>	21801							
45	<i>Brevibacterium</i>	<i>Lactofermentum</i>			B11470					
	<i>Brevibacterium</i>	<i>Lactofermentum</i>			B11471					
	<i>Brevibacterium</i>	<i>Lactofermentum</i>	21086							

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	Genus	Species	ATCC	FERM	NRRL	CECT	NCIMB	CBS	NCTC	DSMZ
	Brevibacterium	Lactofermentum	21420							
	Brevibacterium	Lactofermentum	21086							
	Brevibacterium	Lactofermentum	31269							
5	Brevibacterium	Linens	9174							
	Brevibacterium	Linens	19391							
	Brevibacterium	Linens	8377							
	Brevibacterium	Paraffinolyticum					11160			
	Brevibacterium	spec.						717.73		
	Brevibacterium	spec.						717.73		
10	Brevibacterium	spec.	14604							
	Brevibacterium	spec.	21860							
	Brevibacterium	spec.	21864							
	Brevibacterium	spec.	21865							
	Brevibacterium	spec.	21866							
	Brevibacterium	spec.	19240							
15	Corynebacterium	Acetoacidophilum	21476							
	Corynebacterium	Acetoacidophilum	13870							
	Corynebacterium	Acetoglutamicum			B11473					
20	Corynebacterium	Acetoglutamicum			B11475					
	Corynebacterium	Acetoglutamicum	15806							
	Corynebacterium	Acetoglutamicum	21491							
25	Corynebacterium	Acetoglutamicum	31270							
	Corynebacterium	Acetophilum			B3671					
	Corynebacterium	Ammoniagenes	6872						2399	
30	Corynebacterium	Ammoniagenes	15511							
	Corynebacterium	Fujiokense	21496							
	Corynebacterium	Glutamicum	14067							
35	Corynebacterium	Glutamicum	39137							
	Corynebacterium	Glutamicum	21254							
	Corynebacterium	Glutamicum	21255							
40	Corynebacterium	Glutamicum	31830							
	Corynebacterium	Glutamicum	13032							
	Corynebacterium	Glutamicum	14305							
45	Corynebacterium	Glutamicum	15455							
	Corynebacterium	Glutamicum	13058							

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	Genus	Species	ATCC	FERM	NRRL	CECT	NCIMB	CBS	NCTC	DSMZ
5	Coryne- bacterium	Glutamicum	13059							
	Coryne- bacterium	Glutamicum	13060							
	Coryne- bacterium	Glutamicum	21492							
	Coryne- bacterium	Glutamicum	21513							
	Coryne- bacterium	Glutamicum	21526							
10	Coryne- bacterium	Glutamicum	21543							
	Coryne- bacterium	Glutamicum	13287							
	Coryne- bacterium	Glutamicum	21851							
15	Coryne- bacterium	Glutamicum	21253							
	Coryne- bacterium	glutamicum	21514							
	Coryne- bacterium	glutamicum	21516							
20	Coryne- bacterium	glutamicum	21299							
	Coryne- bacterium	glutamicum	21300							
	Coryne- bacterium	glutamicum	39684							
25	Coryne- bacterium	glutamicum	21488							
	Coryne- bacterium	glutamicum	21649							
	Coryne- bacterium	glutamicum	21650							
30	Coryne- bacterium	glutamicum	19223							
	Coryne- bacterium	glutamicum	13869							
	Coryne- bacterium	glutamicum	21157							
35	Coryne- bacterium	glutamicum	21158							
	Coryne- bacterium	glutamicum	21159							
	Coryne- bacterium	glutamicum	21355							
40	Coryne- bacterium	glutamicum	31808							
	Coryne- bacterium	glutamicum	21674							
	Coryne- bacterium	glutamicum	21562							
45	Coryne- bacterium	glutamicum	21563							
	Coryne- bacterium	glutamicum	21564							

	Genus	Species	ATCC	FERM	NRRL	CECT	NCIMB	CBS	NCTC	DSMZ
5	Coryne- bacterium	glutamicum	21565							
	Coryne- bacterium	glutamicum	21566							
	Coryne- bacterium	glutamicum	21567							
	Coryne- bacterium	glutamicum	21568							
10	Coryne- bacterium	glutamicum	21569							
	Coryne- bacterium	glutamicum	21570							
	Coryne- bacterium	glutamicum	21571							
	Coryne- bacterium	glutamicum	21572							
15	Coryne- bacterium	glutamicum	21573							
	Coryne- bacterium	glutamicum	21579							
	Coryne- bacterium	glutamicum	19049							
	Coryne- bacterium	glutamicum	19050							
20	Coryne- bacterium	glutamicum	19051							
	Coryne- bacterium	glutamicum	19052							
	Coryne- bacterium	glutamicum	19053							
	Coryne- bacterium	glutamicum	19054							
25	Coryne- bacterium	glutamicum	19055							
	Coryne- bacterium	glutamicum	19056							
	Coryne- bacterium	glutamicum	19057							
	Coryne- bacterium	glutamicum	19058							
30	Coryne- bacterium	glutamicum	19059							
	Coryne- bacterium	glutamicum	19060							
	Coryne- bacterium	glutamicum	19185							
	Coryne- bacterium	glutamicum	13286							
35	Coryne- bacterium	glutamicum	21515							
	Coryne- bacterium	glutamicum	21527							
	Coryne- bacterium	glutamicum	21544							
	Coryne- bacterium	glutamicum	21492							

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	Genus	Species	ATCC	FERM	NRRL	CECT	NCIMB	CBS	NCTC	DSMZ
5	Coryne- bacterium	glutamicum			B8183					
	Coryne- bacterium	glutamicum			B8182					
	Coryne- bacterium	glutamicum			B12416					
	Coryne- bacterium	glutamicum			B12417					
	Coryne- bacterium	glutamicum			B12418					
10	Coryne- bacterium	glutamicum			B11476					
	Coryne- bacterium	glutamicum	21608							
	Coryne- bacterium	lilium		P973						
15	Coryne- bacterium	nitrilophilus	21419				11594			
	Coryne- bacterium	spec.		P4445						
	Coryne- bacterium	spec.		P4446						
20	Coryne- bacterium	spec.	31088							
	Coryne- bacterium	spec.	31089							
	Coryne- bacterium	spec.	31090							
25	Coryne- bacterium	spec.	31090							
	Coryne- bacterium	spec.	31090							
	Coryne- bacterium	spec.	15954							20145
30	Coryne- bacterium	spec.	21857							
	Coryne- bacterium	spec.	21862							
	Coryne- bacterium	spec.	21863							

35

ATCC: American Type Culture Collection, Rockville, MD, USA

FERM: Fermentation Research Institute, Chiba, Japan

NRRL: ARS Culture Collection, Northern Regional Research Laboratory, Peoria, IL, USA

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CECT: Coleccion Espanola de Cultivos Tipo, Valencia, Spain

NCIMB: National Collection of Industrial and Marine Bacteria Ltd., Aberdeen, UK

CBS: Centraalbureau voor Schimmelcultures, Baarn, NL

NCTC: National Collection of Type Cultures, London, UK

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DSMZ: Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany

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- Another aspect of the invention pertains to methods for modulating production of a molecule from a microorganism. Such methods include contacting the cell with an agent which modulates MP protein activity or MP nucleic acid expression such that
- 5 a cell associated activity is altered relative to this same activity in the absence of the agent. In a preferred embodiment, the cell is modulated for one or more *C. glutamicum* amino acid, vitamin, cofactor, nutraceutical, nucleotide, nucleoside, or trehalose metabolic pathways, such that the yields or rate of
- 10 production of a desired fine chemical by this microorganism is improved. The agent which modulates MP protein activity can be an agent which stimulates MP protein activity or MP nucleic acid expression. Examples of agents which stimulate MP protein activity or MP nucleic acid expression include small molecules,
- 15 active MP proteins, and nucleic acids encoding MP proteins that have been introduced into the cell. Examples of agents which inhibit MP activity or expression include small molecules, and antisense MP nucleic acid molecules.
- 20 Another aspect of the invention pertains to methods for modulating yields of a desired compound from a cell, involving the introduction of a wild-type or mutant MP gene into a cell, either maintained on a separate plasmid or integrated into the genome of the host cell. If integrated into the genome, such
- 25 integration can be random, or it can take place by homologous recombination such that the native gene is replaced by the introduced copy, causing the production of the desired compound from the cell to be modulated. In a preferred embodiment, said yields are increased. In another preferred embodiment,
- 30 said chemical is a fine chemical. In a particularly preferred embodiment, said fine chemical is trehalose or an amino acid. In especially preferred embodiments, said amino acid are L-lysine and L-methionine.

35 Detailed Description of the Invention

- The present invention provides MP nucleic acid and protein molecules which are involved in the metabolism of certain fine chemicals in *Corynebacterium glutamicum*, including amino acids,
- 40 vitamins, cofactors, nutraceuticals, nucleotides, nucleosides, and trehalose. The molecules of the invention may be utilized in the modulation of production of fine chemicals from microorganisms, such as *C. glutamicum*, either directly (e.g., where modulation of the activity of a trehalose or a lysine
- 45 or methionine biosynthesis protein has a direct impact on the production or efficiency of production of trehalose or lysine or methionine from that organism), or may have an indirect impact

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which nonetheless results in an increase of yield or efficiency of production of the desired compound (e.g., where modulation of the activity of a nucleotide biosynthesis protein has an impact on the production of an organic acid or a fatty acid from

5 the bacterium, perhaps due to improved growth or an increased supply of necessary co-factors, energy compounds, or precursor molecules). Aspects of the invention are further explicated below.

10 1. Fine Chemicals

The term 'fine chemical' is art-recognized and includes molecules produced by an organism which have applications in various industries, such as, but not limited to, the pharmaceutical, 15 agriculture, and cosmetics industries. Such compounds include organic acids, such as tartaric acid, itaconic acid, and diaminopimelic acid, both proteinogenic and non-proteinogenic amino acids, purine and pyrimidine bases, nucleosides, and nucleotides (as described e.g. in Kuninaka, A. (1996) Nucleotides 20 and related compounds, p. 561-612, in Biotechnology vol. 6, Rehm et al., eds. VCH: Weinheim, and references contained therein), lipids, both saturated and unsaturated fatty acids (e.g., arachidonic acid), diols (e.g., propane diol, and butane diol), carbohydrates (e.g., hyaluronic acid and trehalose), aromatic 25 compounds (e.g., aromatic amines, vanillin, and indigo), vitamins and cofactors (as described in Ullmann's Encyclopedia of Industrial Chemistry, vol. A27, "Vitamins", p. 443-613 (1996) VCH: Weinheim and references therein; and Ong, A.S., Niki, E. & Packer, L. (1995) "Nutrition, Lipids, Health, and Disease" 30 Proceedings of the UNESCO/Confederation of Scientific and Technological Associations in Malaysia, and the Society for Free Radical Research - Asia, held Sept. 1-3, 1994 at Penang, Malaysia, AOCS Press, (1995)), enzymes, polyketides (Cane et al. (1998) *Science* 282: 63-68), and all other chemicals described in 35 Gutcho (1983) *Chemicals by Fermentation*, Noyes Data Corporation, ISBN: 0818805086 and references therein. The metabolism and uses of certain of these fine chemicals are further explicated below.

A. Amino Acid Metabolism and Uses

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Amino acids comprise the basic structural units of all proteins, and as such are essential for normal cellular functioning in all organisms. The term "amino acid" is art-recognized. The proteinogenic amino acids, of which there are 20 species, serve 45 as structural units for proteins, in which they are linked by peptide bonds, while the nonproteinogenic amino acids (hundreds of which are known) are not normally found in proteins (see

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- Ulmann's Encyclopedia of Industrial Chemistry, vol. A2, p. 57-97 VCH: Weinheim (1985)). Amino acids may be in the D- or L- optical configuration, though L-amino acids are generally the only type found in naturally-occurring proteins. Biosynthetic and
- 5 degradative pathways of each of the 20 proteinogenic amino acids have been well characterized in both prokaryotic and eukaryotic cells (see, for example, Stryer, L. Biochemistry, 3rd edition, pages 578-590 (1988)). The 'essential' amino acids (histidine, isoleucine, leucine, lysine, methionine, phenylalanine,
- 10 threonine, tryptophan, and valine), so named because they are generally a nutritional requirement due to the complexity of their biosyntheses, are readily converted by simple biosynthetic pathways to the remaining 11 'nonessential' amino acids (alanine, arginine, asparagine, aspartate, cysteine, glutamate, glutamine,
- 15 glycine, proline, serine, and tyrosine). Higher animals do retain the ability to synthesize some of these amino acids, but the essential amino acids must be supplied from the diet in order for normal protein synthesis to occur.
- 20 Aside from their function in protein biosynthesis, these amino acids are interesting chemicals in their own right, and many have been found to have various applications in the food, feed, chemical, cosmetics, agriculture, and pharmaceutical industries. Lysine is an important amino acid in the nutrition not only of
- 25 humans, but also of monogastric animals such as poultry and swine. Glutamate is most commonly used as a flavor additive (mono-sodium glutamate, MSG) and is widely used throughout the food industry, as are aspartate, phenylalanine, glycine, and cysteine. Glycine, L-methionine and tryptophan are all utilized
- 30 in the pharmaceutical industry. Glutamine, valine, leucine, isoleucine, histidine, arginine, proline, serine and alanine are of use in both the pharmaceutical and cosmetics industries. Threonine, tryptophan, and D/ L-methionine are common feed additives. (Leuchtenberger, W. (1996) Amino acids - technical
- 35 production and use, p. 466-502 in Rehm et al. (eds.) Biotechnology vol. 6, chapter 14a, VCH: Weinheim). Additionally, these amino acids have been found to be useful as precursors for the synthesis of synthetic amino acids and proteins, such as N-acetylcysteine, S-carboxymethyl-L-cysteine,
- 40 (S)-5-hydroxytryptophan, and others described in Ulmann's Encyclopedia of Industrial Chemistry, vol. A2, p. 57-97, VCH: Weinheim, 1985.

- The biosynthesis of these natural amino acids in organisms
- 45 capable of producing them, such as bacteria, has been well characterized (for review of bacterial amino acid biosynthesis and regulation thereof, see Umbarger, H.E. (1978) Ann. Rev.

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Biochem. 47: 533-606). Glutamate is synthesized by the reductive amination of α -ketoglutarate, an intermediate in the citric acid cycle. Glutamine, proline, and arginine are each subsequently produced from glutamate. The biosynthesis of serine is a
5 three-step process beginning with 3-phosphoglycerate (an intermediate in glycolysis), and resulting in this amino acid after oxidation, transamination, and hydrolysis steps. Both cysteine and glycine are produced from serine; the former by the condensation of homocysteine with serine, and the latter by the
10 transferal of the side-chain α -carbon atom to tetrahydrofolate, in a reaction catalyzed by serine transhydroxymethylase. Phenylalanine, and tyrosine are synthesized from the glycolytic and pentose phosphate pathway precursors erythrose 4-phosphate and phosphoenolpyruvate in a 9-step biosynthetic pathway that
15 differ only at the final two steps after synthesis of prephenate. Tryptophan is also produced from these two initial molecules, but its synthesis is an 11-step pathway. Tyrosine may also be synthesized from phenylalanine, in a reaction catalyzed by phenylalanine hydroxylase. Alanine, valine, and leucine are all
20 biosynthetic products of pyruvate, the final product of glycolysis. Aspartate is formed from oxaloacetate, an intermediate of the citric acid cycle. Asparagine, methionine, threonine, and lysine are each produced by the conversion of aspartate. Isoleucine is formed from threonine. The biosynthetic
25 pathways leading to methionine have been studied in diverse organisms and show similarity as well as differences. The first step, acylation of homoserine, is common to all the organisms, even though the source of the transferred acyl groups is different. *Escherichia coli* and the related species use
30 succinyl-CoA (Michaeli, S. and Ron, E. Z. (1981) Construction and physical mapping of plasmids containing the *metA* gene of *Escherichia coli* K12, *Mol. Gen. Genet.* **182**, 349-354). Construction and physical mapping of plasmids containing the *metA* gene of *Escherichia coli* K12, *Mol. Gen. Genet.* **182**, 349-354),
35 while *Saccharomyces cerevisiae* (Langin, T., Faugeron, G., Goyon, C., Nicolas, A., and Rossignol, J. (1986) The MET2 gene of *Saccharomyces cerevisiae*: molecular cloning and nucleotide sequence. *Gene* **49**, 283-293), *Brevibacterium flavum* (Miyajima, R. and Shio, I. (1973) Regulation of aspartate family of amino acid
40 biosynthesis in *Brevibacterium flavum*: properties of homoserine O-transacetylase. *J. Biochem.* **73**, 1061-1068; Ozaki, H. and Shio, I. (1982) Methionine biosynthesis in *Brevibacterium flavum*: properties and essential role of O-acetylhomoserine sulfhydrylase. *J. Biochem.* **91**, 1163-1171), *C. glutamicum* (Park, S.-D., Lee, J.-Y., Kim, Y., Kim, J.-H., and Lee, H.-S. (1998)
45 Isolation and analysis of *metA*, a methionine biosynthetic gene encoding homoserine acetyltransferase in *Corynebacterium*

- glutamicum*. *Mol. Cells* **8**, 286-294), and *Leptospira meyeri* (Belfaiza, J., Martel, A., Maegarita, D., and Saint Girons, I. (1998) Direct sulfhydrylation for methionine biosynthesis in *Leptospira meyeri*. *J. Bacteriol.* **180**, 250-255; Bourhy, P.,
- 5 Martel, A., Margarita, D., Saint Girons, I., and Belfaiza, J. (1997) Homoserine O-acetyltransferase, involved in the *Leptospira meyeri* methionine biosynthetic pathway, is not feedback inhibited. *J. Bacteriol.* **179**, 4396-4398) use acetyl-CoA as the acyl donor. Formation of homocysteine from acylhomoserine can
- 10 occur in two different ways. *E. coli* uses the transsulfuration pathway which is catalyzed by cystathionine γ -synthase (the product of *metB*) and cystathionine β -lyase (the product of *metC*). *S. cerevisiae* (Cherest, H. and Surdin-Kerjan, Y. (1992) Genetic analysis of a new mutation conferring cysteine auxotrophy in
- 15 *Saccharomyces cerevisiae*: updating of the sulfur metabolism pathway. *Genetics* **130**, 51-58), *B. flavum* (Ozaki, H. and Shio, I. (1982) Methionine biosynthesis in *Brevibacterium flavum*: properties and essential role of O-acetylhomoserine
- 20 sulfhydrylase. *J. Biochem.* **91**, 1163-1171), *Pseudomonas aeruginosa* (Fogliano, M., Borne, F., Bally, M., Ball, G., and Patte, J. C. (1995) A direct sulfhydrylation pathway is used for methionine biosynthesis in *Pseudomonas aeruginosa*. *Microbiology* **141**, 431-439), and *L. meyeri* (Belfaiza, J., Martel, A., Maegarita, D., and Saint Girons, I. (1998) Direct sulfhydrylation for methionine
- 25 biosynthesis in *Leptospira meyeri*. *J. Bacteriol.* **180**, 250-255) utilize the direct sulfhydrylation pathway which is catalyzed by acylhomoserine sulfhydrylase. Unlike closely related *B. flavum* which uses only the direct sulfhydrylation pathway, enzyme activities of the transsulfuration pathway have been detected in
- 30 the extracts of the *C. glutamicum* cells and the pathway has been proposed to be the route for methionine biosynthesis in the organism (Hwang, B.-J., Kim, Y., Kim, H.-B., Kim, J., Hwang, H.-J., and Lee, H.-S. (1999) Analysis of *Corynebacterium glutamicum* methionine biosynthetic pathway: Isolation and
- 35 analysis of *metB* encoding cystathionine α -synthase. *Mol. Cells* **9**, 300-308; Kase, H. and Nakayama, K. (1974) Production of O-acetyl-L-homoserine by methionine analog resistant mutants and regulation of homoserine-O-transacetylase in *Corynebacterium glutamicum*. *Agr. Biol. Chem.* **38**, 2021-2030; Park, S.-D., Lee,
- 40 J.-Y., Kim, Y., Kim, J.-H., and Lee, H.-S. (1998) Isolation and analysis of *metA*, a methionine biosynthetic gene encoding homoserine acetyltransferase in *Corynebacterium glutamicum*. *Mol. Cells* **8**, 286-294).
- 45 Even though some genes involved in methionine biosynthesis in *C. glutamicum* were isolated in recent years, the information on the biosynthesis of methionine in *C. glutamicum* is still limited.

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The metA and metB genes have been isolated from the organism and also the metC and the metZ gene are known (table 4), but the final step of the biosynthesis remained unclear. In this invention, the biosynthetic pathway leading to methionine in
5 *C. glutamicum* is deciphered in total and the biosynthetic gene responsible for the last step of the biosynthesis is defined with the metH gene encoding the enzyme methionine synthase.

A complex 9-step pathway results in the production of histidine
10 from 5-phosphoribosyl-1-pyrophosphate, an activated sugar.

Amino acids in excess of the protein synthesis needs of the cell cannot be stored, and are instead degraded to provide intermediates for the major metabolic pathways of the cell (for
15 review see Stryer, L. Biochemistry 3rd ed. Ch. 21 "Amino Acid Degradation and the Urea Cycle" p. 495-516 (1988)). Although the cell is able to convert unwanted amino acids into useful metabolic intermediates, amino acid production is costly in terms of energy, precursor molecules, and the enzymes necessary to
20 synthesize them. Thus it is not surprising that amino acid biosynthesis is regulated by feedback inhibition, in which the presence of a particular amino acid serves to slow or entirely stop its own production (for overview of feedback mechanisms in amino acid biosynthetic pathways, see Stryer, L. Biochemistry,
25 3rd ed. Ch. 24: "Biosynthesis of Amino Acids and Heme" p. 575-600 (1988)). Thus, the output of any particular amino acid is limited by the amount of that amino acid present in the cell.

2.1 Vitamin, Cofactor, and Nutraceutical Metabolism and Uses

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Vitamins, cofactors, and nutraceuticals comprise another group of molecules which the higher animals have lost the ability to synthesize and so must ingest, although they are readily synthesized by other organisms, such as bacteria. These molecules
35 are either bioactive substances themselves, or are precursors of biologically active substances which may serve as electron carriers or intermediates in a variety of metabolic pathways. Aside from their nutritive value, these compounds also have significant industrial value as coloring agents, antioxidants,
40 and catalysts or other processing aids. (For an overview of the structure, activity, and industrial applications of these compounds, see, for example, Ullman's Encyclopedia of Industrial Chemistry, "Vitamins" vol. A27, p. 443-613, VCH: Weinheim, 1996.) The term "vitamin" is art-recognized, and includes nutrients
45 which are required by an organism for normal functioning, but which that organism cannot synthesize by itself. The group of vitamins may encompass cofactors and nutraceutical compounds. The

- language "cofactor" includes nonproteinaceous compounds required for a normal enzymatic activity to occur. Such compounds may be organic or inorganic; the cofactor molecules of the invention are preferably organic. The term "nutraceutical" includes dietary
- 5 supplements having health benefits in plants and animals, particularly humans. Examples of such molecules are vitamins, antioxidants, and also certain lipids (e.g., polyunsaturated fatty acids).
- 10 The biosynthesis of these molecules in organisms capable of producing them, such as bacteria, has been largely characterized (Ullman's Encyclopedia of Industrial Chemistry, "Vitamins" vol. A27, p. 443-613, VCH: Weinheim, 1996; Michal, G. (1999) Biochemical Pathways: An Atlas of Biochemistry and Molecular
- 15 Biology, John Wiley & Sons; Ong, A.S., Niki, E. & Packer, L. (1995) "Nutrition, Lipids, Health, and Disease" Proceedings of the UNESCO/Confederation of Scientific and Technological Associations in Malaysia, and the Society for Free Radical Research - Asia, held Sept. 1-3, 1994 at Penang, Malaysia,
- 20 AOCs Press: Champaign, IL X, 374 S).

- Thiamin (vitamin B₁) is produced by the chemical coupling of pyrimidine and thiazole moieties. Riboflavin (vitamin B₂) is synthesized from guanosine-5'-triphosphate (GTP) and
- 25 ribose-5'-phosphate. Riboflavin, in turn, is utilized for the synthesis of flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD). The family of compounds collectively termed 'vitamin B₆' (e.g., pyridoxine, pyridoxamine, pyridoxa-5'-phosphate, and the commercially used pyridoxin
- 30 hydrochloride) are all derivatives of the common structural unit, 5-hydroxy-6-methylpyridine. Pantothenate (pantothenic acid, (R)-(+)-N-(2,4-dihydroxy-3,3-dimethyl-1-oxobutyl)- α -alanine) can be produced either by chemical synthesis or by fermentation. The final steps in pantothenate biosynthesis consist of the
- 35 ATP-driven condensation of α -alanine and pantoic acid. The enzymes responsible for the biosynthesis steps for the conversion to pantoic acid, to α -alanine and for the condensation to pantothenic acid are known. The metabolically active form of pantothenate is Coenzyme A, for which the biosynthesis proceeds
- 40 in 5 enzymatic steps. Pantothenate, pyridoxal-5'-phosphate, cysteine and ATP are the precursors of Coenzyme A. These enzymes not only catalyze the formation of pantothenate, but also the production of (R)-pantoic acid, (R)-pantolacton, (R)-panthenol (provitamin B₅), pantetheine (and its derivatives) and coenzyme A.

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Biotin biosynthesis from the precursor molecule pimeloyl-CoA in microorganisms has been studied in detail and several of the genes involved have been identified. Many of the corresponding proteins have been found to also be involved in Fe-cluster synthesis and are members of the nifS class of proteins. Lipoic acid is derived from octanoic acid, and serves as a coenzyme in energy metabolism, where it becomes part of the pyruvate dehydrogenase complex and the α -ketoglutarate dehydrogenase complex. The folates are a group of substances which are all derivatives of folic acid, which in turn is derived from L-glutamic acid, p-amino-benzoic acid and 6-methylpterin. The biosynthesis of folic acid and its derivatives, starting from metabolism intermediates guanosine-5'-triphosphate (GTP), L-glutamic acid and p-amino-benzoic acid has been studied in detail in certain microorganisms.

Corrinoids (such as the cobalamines and particularly vitamin B₁₂) and porphyrines belong to a group of chemicals characterized by a tetrapyrrole ring system. The biosynthesis of vitamin B₁₂ is sufficiently complex that it has not yet been completely characterized, but many of the enzymes and substrates involved are now known. Nicotinic acid (nicotinate), and nicotinamide are pyridine derivatives which are also termed 'niacin'. Niacin is the precursor of the important coenzymes NAD (nicotinamide adenine dinucleotide) and NADP (nicotinamide adenine dinucleotide phosphate) and their reduced forms.

The large-scale production of these compounds has largely relied on cell-free chemical syntheses, though some of these chemicals have also been produced by large-scale culture of microorganisms, such as riboflavin, Vitamin B₆, pantothenate, and biotin. Only Vitamin B₁₂ is produced solely by fermentation, due to the complexity of its synthesis. In vitro methodologies require significant inputs of materials and time, often at great cost.

35 C. Purine, Pyrimidine, Nucleoside and Nucleotide Metabolism and Uses

Purine and pyrimidine metabolism genes and their corresponding proteins are important targets for the therapy of tumor diseases and viral infections. The language "purine" or "pyrimidine" includes the nitrogenous bases which are constituents of nucleic acids, co-enzymes, and nucleotides. The term "nucleotide" includes the basic structural units of nucleic acid molecules, which are comprised of a nitrogenous base, a pentose sugar (in the case of RNA, the sugar is ribose; in the case of DNA, the sugar is D-deoxyribose), and phosphoric acid. The language

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"nucleoside" includes molecules which serve as precursors to nucleotides, but which are lacking the phosphoric acid moiety that nucleotides possess. By inhibiting the biosynthesis of these molecules, or their mobilization to form nucleic acid molecules, it is possible to inhibit RNA and DNA synthesis; by inhibiting this activity in a fashion targeted to cancerous cells, the ability of tumor cells to divide and replicate may be inhibited. Additionally, there are nucleotides which do not form nucleic acid molecules, but rather serve as energy stores (*i.e.*, AMP) or as coenzymes (*i.e.*, FAD and NAD).

Several publications have described the use of these chemicals for these medical indications, by influencing purine and/or pyrimidine metabolism (*e.g.* Christopherson, R.I. and Lyons, S.D. (1990) "Potent inhibitors of *de novo* pyrimidine and purine biosynthesis as chemotherapeutic agents." *Med. Res. Reviews* 10: 505-548). Studies of enzymes involved in purine and pyrimidine metabolism have been focused on the development of new drugs which can be used, for example, as immunosuppressants or anti-proliferants (Smith, J.L., (1995) "Enzymes in nucleotide synthesis." *Curr. Opin. Struct. Biol.* 5: 752-757; (1995) *Biochem Soc. Transact.* 23: 877-902). However, purine and pyrimidine bases, nucleosides and nucleotides have other utilities: as intermediates in the biosynthesis of several fine chemicals (*e.g.*, thiamine, S-adenosyl-methionine, folates, or riboflavin), as energy carriers for the cell (*e.g.*, ATP or GTP), and for chemicals themselves, commonly used as flavor enhancers (*e.g.*, IMP or GMP) or for several medicinal applications (see, for example, Kuninaka, A. (1996) *Nucleotides and Related Compounds in Biotechnology* vol. 6, Rehm et al., eds. VCH: Weinheim, p. 561-612). Also, enzymes involved in purine, pyrimidine, nucleoside, or nucleotide metabolism are increasingly serving as targets against which chemicals for crop protection, including fungicides, herbicides and insecticides, are developed.

The metabolism of these compounds in bacteria has been characterized (for reviews see, for example, Zalkin, H. and Dixon, J.E. (1992) "*de novo* purine nucleotide biosynthesis", in: *Progress in Nucleic Acid Research and Molecular Biology*, vol. 42, Academic Press:, p. 259-287; and Michal, G. (1999) "Nucleotides and Nucleosides", Chapter 8 in: *Biochemical Pathways: An Atlas of Biochemistry and Molecular Biology*, Wiley: New York). Purine metabolism has been the subject of intensive research, and is essential to the normal functioning of the cell. Impaired purine metabolism in higher animals can cause severe disease, such as gout. Purine nucleotides are synthesized from ribose-5-phosphate, in a series of steps through the intermediate compound

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inosine-5'-phosphate (IMP), resulting in the production of guanosine-5'-monophosphate (GMP) or adenosine-5'-monophosphate (AMP), from which the triphosphate forms utilized as nucleotides are readily formed. These compounds are also utilized as energy stores, so their degradation provides energy for many different biochemical processes in the cell. Pyrimidine biosynthesis proceeds by the formation of uridine-5'-monophosphate (UMP) from ribose-5-phosphate. UMP, in turn, is converted to cytidine-5'-triphosphate (CTP). The deoxy- forms of all of these nucleotides are produced in a one step reduction reaction from the diphosphate ribose form of the nucleotide to the diphosphate deoxyribose form of the nucleotide. Upon phosphorylation, these molecules are able to participate in DNA synthesis.

15 D. Trehalose Metabolism and Uses

Trehalose consists of two glucose molecules, bound in a α, α -1,1 linkage. It is commonly used in the food industry as a sweetener, an additive for dried or frozen foods, and in beverages. However, it also has applications in the pharmaceutical, cosmetics and biotechnology industries (see, for example, Nishimoto et al., (1998) U.S. Patent No. 5,759,610; Singer, M.A. and Lindquist, S. (1998) *Trends Biotech.* 16: 460-467; Paiva, C.L.A. and Panek, A.D. (1996) *Biotech. Ann. Rev.* 2: 293-314; and Shiosaka, M. (1997) *J. Japan* 172: 97-102). Trehalose is produced by enzymes from many microorganisms and is naturally released into the surrounding medium, from which it can be collected using methods known in the art.

30 II. Elements and Methods of the Invention

The present invention is based, at least in part, on the discovery of novel molecules, referred to herein as MP nucleic acid and protein molecules, which play a role in or function in one or more cellular metabolic pathways. In one embodiment, the MP molecules catalyze an enzymatic reaction involving one or more amino acid, vitamin, cofactor, nutraceutical, nucleotide, nucleoside, or trehalose metabolic pathways. In a preferred embodiment, the activity of the MP molecules of the present invention in one or more *C. glutamicum* metabolic pathways for amino acids, vitamins, cofactors, nutraceuticals, nucleotides, nucleosides or trehalose has an impact on the production of a desired fine chemical by this organism. In a particularly preferred embodiment, the MP molecules of the invention are modulated in activity, such that the *C. glutamicum* metabolic pathways in which the MP proteins of the invention are involved are modulated in efficiency or output, which either directly or

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- indirectly modulates the production or efficiency of production of a desired fine chemical by *C. glutamicum*. The MP molecules may be combined with other MP molecules of the same or different metabolic pathway to increase the yield of a desired fine chemical, preferred trehalose or an amino acid, more preferred lysine or methionine. Alternatively or in addition a byproduct which is not desired may be reduced by combination of disruption of MP molecules or other metabolic molecules. The MP molecules combined with other MP molecules of the same or a different pathway may be altered in their nucleotide and in the corresponding amino acid sequence in such a way that their activity is altered under physiological conditions which leads to an increase in productivity and/or yield of a desired fine chemical. In a further embodiment the MP molecule in its original or in its above described altered form may be combined with other MP molecules of the same or a different pathway which are altered in their nucleotide sequence in such a way that their activity is altered under physiological conditions which leads to an increase in productivity and/or yield of a desired fine chemical.
- Preferred combinations are such which combine one or both MP molecules of table 1 with one or more single or multiple copies of MP proteins of tables 4 and 5 or the respective published MP molecules of the same metabolic pathway (Methionine biosynthesis or trehalose/phosphoenolpyruvate way).
- The language, "MP protein" or "MP polypeptide" includes proteins which play a role in, e.g., catalyze an enzymatic reaction, in one or more amino acid, vitamin, cofactor, nutraceutical, nucleotide, nucleoside or trehalose metabolic pathways. Examples of MP proteins include those encoded by the MP genes set forth in Table 1 and by the odd-numbered SEQ ID NOs. The terms "MP gene" or "MP nucleic acid sequence" include nucleic acid sequences encoding an MP protein, which consist of a coding region and also corresponding untranslated 5' and 3' sequence regions. Examples of MP genes include those set forth in Table 1. The terms "production" or "productivity" are art-recognized and include the concentration of the fermentation product (for example, the desired fine chemical) formed within a given time and a given fermentation volume (e.g., kg product per hour per liter). The term "efficiency of production" includes the time required for a particular level of production to be achieved (for example, how long it takes for the cell to attain a particular rate of output of a fine chemical). The term "yield" or "product/carbon yield" is art-recognized and includes the efficiency of the conversion of the carbon source into the product (i.e., fine chemical). This is generally written as, for example, kg product per kg carbon source. By increasing the yield or production of the compound,

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the quantity of recovered molecules, or of useful recovered molecules of that compound in a given amount of culture over a given amount of time is increased. The terms "biosynthesis" or a "biosynthetic pathway" are art-recognized and include the
5 synthesis of a compound, preferably an organic compound, by a cell from intermediate compounds in what may be a multistep and highly regulated process. The terms "degradation" or a "degradation pathway" are art-recognized and include the
10 breakdown of a compound, preferably an organic compound, by a cell to degradation products (generally speaking, smaller or less complex molecules) in what may be a multistep and highly regulated process. The language "metabolism" is art-recognized and includes the totality of the biochemical reactions that take place in an organism. The metabolism of a particular compound,
15 then, (e.g., the metabolism of an amino acid such as glycine) comprises the overall biosynthetic, modification, and degradation pathways in the cell related to this compound.

In another embodiment, the MP molecules of the invention are
20 capable of modulating the production of a desired molecule, such as a fine chemical, in a microorganism such as *C. glutamicum*. Using recombinant genetic techniques, one or more of the biosynthetic or degradative enzymes of the invention for amino acids, vitamins, cofactors, nutraceuticals, nucleotides,
25 nucleosides, or trehalose may be manipulated such that its function is modulated. For example, a biosynthetic enzyme may be improved in efficiency, or its allosteric control region destroyed such that feedback inhibition of production of the compound is prevented. Similarly, a degradative enzyme may
30 be deleted or modified by substitution, deletion, or addition such that its degradative activity is lessened for the desired compound without impairing the viability of the cell. In each case, the overall yield or rate of production of one of these desired fine chemicals may be increased.

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It is also possible that such alterations in the protein and nucleotide molecules of the invention may improve the production of other fine chemicals besides the amino acids, vitamins, cofactors, nutraceuticals, nucleotides, nucleosides, and
40 trehalose. Metabolism of any one compound is necessarily intertwined with other biosynthetic and degradative pathways within the cell, and necessary cofactors, intermediates, or substrates in one pathway are likely supplied or limited by another such pathway. Therefore, by modulating the activity of
45 one or more of the proteins of the invention, the production or efficiency of activity of another fine chemical biosynthetic or degradative pathway may be impacted. For example, amino acids

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serve as the structural units of all proteins, yet may be present intracellularly in levels which are limiting for protein synthesis; therefore, by increasing the efficiency of production or the yields of one or more amino acids within the cell, proteins, such as biosynthetic or degradative proteins, may be more readily synthesized. Likewise, an alteration in a metabolic pathway enzyme such that a particular side reaction becomes more or less favored may result in the over- or under-production of one or more compounds which are utilized as intermediates or substrates for the production of a desired fine chemical.

The isolated nucleic acid sequences of the invention are contained within the genome of a *Corynebacterium glutamicum* strain available through the American Type Culture Collection, given designation ATCC 13032. The nucleotide sequence of the isolated *C. glutamicum* MP DNAs and the predicted amino acid sequences of the *C. glutamicum* MP proteins are shown in the Sequence Listing as odd-numbered SEQ ID NOs and even-numbered SEQ ID NOs, respectively. Computational analyses were performed which classified and/or identified these nucleotide sequences as sequences which encode metabolic pathway proteins.

The present invention also pertains to proteins which have an amino acid sequence which is substantially homologous to an amino acid sequence of the invention (e.g., the sequence of an even-numbered SEQ ID NO of the Sequence Listing). As used herein, a protein which has an amino acid sequence which is substantially homologous to a selected amino acid sequence is least about 50% homologous to the selected amino acid sequence, e.g., the entire selected amino acid sequence. A protein which has an amino acid sequence which is substantially homologous to a selected amino acid sequence can also be least about 50-60%, preferably at least about 60-70%, and more preferably at least about 70-80%, 80-90%, or 90-95%, and most preferably at least about 96%, 97%, 98%, 99% or more homologous to the selected amino acid sequence.

The MP protein or a biologically active portion or fragment thereof of the invention can catalyze an enzymatic reaction in one or more amino acid, vitamin, cofactor, nutraceutical, nucleotide, nucleoside, or trehalose metabolic pathways, or have one or more of the activities set forth in Table 1.

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Various aspects of the invention are described in further detail in the following subsections:

A. Isolated Nucleic Acid Molecules

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One aspect of the invention pertains to isolated nucleic acid molecules that encode MP polypeptides or biologically active portions thereof, as well as nucleic acid fragments sufficient for use as hybridization probes or primers for the identification or amplification of MP-encoding nucleic acid (e.g., MP DNA). As used herein, the term "nucleic acid molecule" is intended to include DNA molecules (e.g., cDNA or genomic DNA) and RNA molecules (e.g., mRNA) and analogs of the DNA or RNA generated using nucleotide analogs. This term also encompasses untranslated sequence located at both the 3' and 5' ends of the coding region of the gene: at least about 100 nucleotides of sequence upstream from the 5' end of the coding region and at least about 20 nucleotides of sequence downstream from the 3' end of the coding region of the gene. The nucleic acid molecule can be single-stranded or double-stranded, but preferably is double-stranded DNA. An "isolated" nucleic acid molecule is one which is separated from other nucleic acid molecules which are present in the natural source of the nucleic acid. Preferably, an "isolated" nucleic acid is free of sequences which naturally flank the nucleic acid (i.e., sequences located at the 5' and 3' ends of the nucleic acid) in the genomic DNA of the organism from which the nucleic acid is derived. For example, in various embodiments, the isolated MP nucleic acid molecule can contain less than about 5 kb, 4kb, 3kb, 2kb, 1 kb, 0.5 kb or 0.1 kb of nucleotide sequences which naturally flank the nucleic acid molecule in genomic DNA of the cell from which the nucleic acid is derived (e.g., a *C. glutamicum* cell). Moreover, an "isolated" nucleic acid molecule, such as a DNA molecule, can be substantially free of other cellular material, or culture medium when produced by recombinant techniques, or chemical precursors or other chemicals when chemically synthesized.

A nucleic acid molecule of the present invention, e.g., a nucleic acid molecule having a nucleotide sequence of an odd-numbered SEQ ID NO of the Sequence Listing, or a portion thereof, can be isolated using standard molecular biology techniques and the sequence information provided herein. For example, a *C. glutamicum* MP DNA can be isolated from a *C. glutamicum* library using all or portion of one of the odd-numbered SEQ ID NO sequences of the Sequence Listing as a hybridization probe and standard hybridization techniques (e.g., as described in Sambrook, J., Fritsh, E. F., and Maniatis, T. *Molecular Cloning*:

A Laboratory Manual. 2nd, ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989). Moreover, a nucleic acid molecule encompassing all or a portion of one of the nucleic acid sequences of the invention

5 (e.g., an odd-numbered SEQ ID NO:) can be isolated by the polymerase chain reaction using oligonucleotide primers designed based upon this sequence (e.g., a nucleic acid molecule encompassing all or a portion of one of the nucleic acid sequences of the invention (e.g., an odd-numbered SEQ ID NO of

10 the Sequence Listing) can be isolated by the polymerase chain reaction using oligonucleotide primers designed based upon this same sequence). For example, mRNA can be isolated from normal endothelial cells (e.g., by the guanidinium-thiocyanate extraction procedure of Chirgwin et al. (1979) *Biochemistry* 18:

15 5294-5299) and DNA can be prepared using reverse transcriptase (e.g., Moloney MLV reverse transcriptase, available from Gibco/BRL, Bethesda, MD; or AMV reverse transcriptase, available from Seikagaku America, Inc., St. Petersburg, FL). Synthetic oligonucleotide primers for polymerase chain reaction

20 amplification can be designed based upon one of the nucleotide sequences shown in the Sequence Listing. A nucleic acid of the invention can be amplified using cDNA or, alternatively, genomic DNA, as a template and appropriate oligonucleotide primers according to standard PCR amplification techniques. The nucleic

25 acid so amplified can be cloned into an appropriate vector and characterized by DNA sequence analysis. Furthermore, oligonucleotides corresponding to an MP nucleotide sequence can be prepared by standard synthetic techniques, e.g., using an automated DNA synthesizer.

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In a preferred embodiment, an isolated nucleic acid molecule of the invention comprises one of the nucleotide sequences shown in the Sequence Listing. The nucleic acid sequences of the invention, as set forth in the Sequence Listing, correspond to

35 the *Corynebacterium glutamicum* MP DNAs of the invention. This DNA comprises sequences encoding MP proteins (i.e., the "coding region", indicated in each odd-numbered SEQ ID NO: sequence in the Sequence Listing), as well as 5' untranslated sequences and 3' untranslated sequences, also indicated in each odd-numbered

40 SEQ ID NO: in the Sequence Listing. Alternatively, the nucleic acid molecule can comprise only the coding region of any of the nucleic acid sequences of the Sequence Listing.

In another preferred embodiment, an isolated nucleic acid

45 molecule of the invention comprises a nucleic acid molecule which is a complement of one of the nucleotide sequences of the invention (e.g., a sequence of an odd-numbered SEQ ID NO: of the

Sequence Listing), or a portion thereof. A nucleic acid molecule which is complementary to one of the nucleotide sequences of the invention is one which is sufficiently complementary to one of the nucleotide sequences shown in the Sequence Listing (e.g.,
5 the sequence of an odd-numbered SEQ ID NO:) such that it can hybridize to one of the nucleotide sequences of the invention, thereby forming a stable duplex.

In still another preferred embodiment, an isolated nucleic acid
10 molecule of the invention comprises a nucleotide sequence which is at least about 63%, 64%, 65%, 66%, 67%, 68%, 69%, or 70%, more preferably at least about 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, or 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, or 90%, or 91%, 92%, 93%, 94%, and even more preferably at least
15 about 95%, 96%, 97%, 98%, 99% or more homologous to a nucleotide sequence of the invention (e.g., a sequence of an odd-numbered SEQ ID NO: of the Sequence Listing), or a portion thereof. Ranges and identity values intermediate to the above-recited ranges, (e.g., 70-90% identical or 80-95% identical) are also intended to
20 be encompassed by the present invention. For example, ranges of identity values using a combination of any of the above values recited as upper and/or lower limits are intended to be included. In an additional preferred embodiment, an isolated nucleic acid molecule of the invention comprises a nucleotide sequence which
25 hybridizes, e.g., hybridizes under stringent conditions, to one of the nucleotide sequences of the invention, or a portion thereof.

Moreover, the nucleic acid molecule of the invention can comprise
30 only a portion of the coding region of the sequence of one of the odd-numbered SEQ ID NOs of the Sequence Listing, for example a fragment which can be used as a probe or primer or a fragment encoding a biologically active portion of an MP protein. The nucleotide sequences determined from the cloning of the MP genes
35 from *C. glutamicum* allows for the generation of probes and primers designed for use in identifying and/or cloning MP homologues in other cell types and organisms, as well as MP homologues from other *Corynebacteria* or related species. The probe/primer typically comprises substantially purified
40 oligonucleotide. The oligonucleotide typically comprises a region of nucleotide sequence that hybridizes under stringent conditions to at least about 12, preferably about 25, more preferably about 40, 50 or 75 consecutive nucleotides of a sense strand of one of the nucleotide sequences of the invention (e.g., a sequence of
45 one of the odd-numbered SEQ ID NOs of the Sequence Listing), an anti-sense sequence of one of these sequences, or naturally occurring mutants thereof. Primers based on a nucleotide sequence

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of the invention can be used in PCR reactions to clone MP homologues. Probes based on the MP nucleotide sequences can be used to detect transcripts or genomic sequences encoding the same or homologous proteins. In preferred embodiments, the probe
5 further comprises a label group attached thereto, e.g. the label group can be a radioisotope, a fluorescent compound, an enzyme, or an enzyme co-factor. Such probes can be used as a part of a diagnostic test kit for identifying cells which misexpress an MP protein, such as by measuring a level of an MP-encoding nucleic
10 acid in a sample of cells from a subject e.g., detecting MP mRNA levels or determining whether a genomic MP gene has been mutated or deleted.

In one embodiment, the nucleic acid molecule of the invention
15 encodes a protein or portion thereof which includes an amino acid sequence which is sufficiently homologous to an amino acid sequence of the invention (e.g., a sequence of an even-numbered SEQ ID NO of the Sequence Listing) such that the protein or portion thereof maintains the ability to catalyze an enzymatic
20 reaction in an amino acid, vitamin, cofactor, nutraceutical, nucleotide, nucleoside, or trehalose metabolic pathway. As used herein, the language "sufficiently homologous" refers to proteins or portions thereof which have amino acid sequences which include a minimum number of identical or equivalent (e.g., an amino acid
25 residue which has a similar side chain as an amino acid residue in a sequence of one of the even-numbered SEQ ID NOs of the Sequence Listing) amino acid residues to an amino acid sequence of the invention such that the protein or portion thereof is able to catalyze an enzymatic reaction in a *C. glutamicum* amino acid,
30 vitamin, cofactor, nutraceutical, nucleotide, nucleoside or trehalose metabolic pathway. Protein members of such metabolic pathways, as described herein, function to catalyze the biosynthesis or degradation of one or more of: amino acids, vitamins, cofactors, nutraceuticals, nucleotides, nucleosides, or
35 trehalose. Examples of such activities are also described herein. Thus, "the function of an MP protein" contributes to the overall functioning of one or more such metabolic pathway and contributes, either directly or indirectly, to the yield, production, and/or efficiency of production of one or more fine
40 chemicals. Examples of MP protein activities are set forth in Table 1.

In another embodiment, the protein is at least about 50-60%, preferably at least about 60-70%, and more preferably at least
45 about 70-80%, 80-90%, 90-95%, and most preferably at least about 96%, 97%, 98%, 99% or more homologous to an entire amino acid

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sequence of the invention (e.g., a sequence of an even-numbered SEQ ID NO: of the Sequence Listing).

Portions of proteins encoded by the MP nucleic acid molecules of the invention are preferably biologically active portions of one of the MP proteins. As used herein, the term "biologically active portion of an MP protein" is intended to include a portion, e.g., a domain/motif, of an MP protein that catalyzes an enzymatic reaction in one or more *C. glutamicum* amino acid, vitamin, cofactor, nutraceutical, nucleotide, nucleoside, or trehalose metabolic pathways, or has an activity as set forth in Table 1. To determine whether an MP protein or a biologically active portion thereof can catalyze an enzymatic reaction in an amino acid, vitamin, cofactor, nutraceutical, nucleotide, nucleoside, or trehalose metabolic pathway, an assay of enzymatic activity may be performed. Such assay methods are well known to those of ordinary skill in the art, as detailed in Example 8 of the Exemplification.

Additional nucleic acid fragments encoding biologically active portions of an MP protein can be prepared by isolating a portion of one of the amino acid sequences of the invention (e.g., a sequence of an even-numbered SEQ ID NO: of the Sequence Listing), expressing the encoded portion of the MP protein or peptide (e.g., by recombinant expression *in vitro*) and assessing the activity of the encoded portion of the MP protein or peptide.

The invention further encompasses nucleic acid molecules that differ from one of the nucleotide sequences of the invention (e.g., a sequence of an odd-numbered SEQ ID NO: of the Sequence Listing) (and portions thereof) due to degeneracy of the genetic code and thus encode the same MP protein as that encoded by the nucleotide sequences of the invention. In another embodiment, an isolated nucleic acid molecule of the invention has a nucleotide sequence encoding a protein having an amino acid sequence shown in the Sequence Listing (e.g., an even-numbered SEQ ID NO:). In a still further embodiment, the nucleic acid molecule of the invention encodes a full length *C. glutamicum* protein which is substantially homologous to an amino acid sequence of the invention (encoded by an open reading frame shown in an odd-numbered SEQ ID NO: of the Sequence Listing).

It will be understood by one of ordinary skill in the art that in one embodiment the sequences of the invention are not meant to include the sequences of the prior art, such as those Genbank sequences set forth in Table 3 which were available prior to the present invention. In one embodiment, the invention includes

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nucleotide and amino acid sequences having a percent identity to a nucleotide or amino acid sequence of the invention which is greater than that of a sequence of the prior art, i.e the invention includes a nucleotide sequence which encodes a protein sequence which is greater than and/or at least 71% identical to the protein sequence designated SEQ ID NO:2 and/or a nucleotide sequence which encodes a protein sequence which is greater than and/or at least 63 % identical to the protein sequence designated SEQ ID NO: 4.

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Table 3: Alignment results

	Gene name (identifier)	Genbank hit	Homology	Reference
15				
	metH	GB_BA2:MTCY261 Mycobacterium tuberculosis H37Rv Complete genome	70.3 %	Cole et al. (1998) Nature 393, 537-544
20				
	treS	GB_BA2:MTCY261 Mycobacterium tuberculosis H37Rv complete genome	62.4 %	Cole et al. (1998) Nature 393, 537-544

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Homology: CLUSTAL-calculated percent identity (Open reading frames from the genome, translated into amino acid sequence)

30 One of ordinary skill in the art would be able to calculate the lower threshold of percent identity for any given sequence of the invention by examining the CLUSTAL-calculated percent identity scores set forth in Table 3 for each of the three top hits for the given sequence. One of ordinary skill in the art will also appreciate that nucleic acid and amino acid sequences having
35 percent identities greater than the lower threshold so calculated (e.g., preferably at least about 63%, 64%, 65%, 66%, 67%, 68%, 69%, or 70%, more preferably at least about 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, or 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, or 90%, or 91%, 92%, 93%, 94%, and even more
40 preferably at least about 95%, 96%, 97%, 98%, 99% or more identical) are also encompassed by the invention.

In addition to the *C. glutamicum* MP nucleotide sequences set forth in the Sequence Listing as odd-numbered SEQ ID NOs, it will
45 be appreciated by one of ordinary skill in the art that DNA sequence polymorphisms that lead to changes in the amino acid sequences of MP proteins may exist within a population (e.g., the

C. glutamicum population). Such genetic polymorphism in the MP gene may exist among individuals within a population due to natural variation. As used herein, the terms "gene" and "recombinant gene" refer to nucleic acid molecules comprising an open reading frame encoding an MP protein, preferably a *C. glutamicum* MP protein. Such natural variations can typically result in 1-5% variance in the nucleotide sequence of the MP gene. Any and all such nucleotide variations and resulting amino acid polymorphisms in MP that are the result of natural variation and that do not alter the functional activity of MP proteins are intended to be within the scope of the invention.

Nucleic acid molecules corresponding to natural variants and non-*C. glutamicum* homologues of the *C. glutamicum* MP DNA of the invention can be isolated based on their homology to the *C. glutamicum* MP nucleic acid disclosed herein using the *C. glutamicum* DNA, or a portion thereof, as a hybridization probe according to standard hybridization techniques under stringent hybridization conditions. Accordingly, in another embodiment, an isolated nucleic acid molecule of the invention is at least 15 nucleotides in length and hybridizes under stringent conditions to the nucleic acid molecule comprising a nucleotide sequence of an odd-numbered SEQ ID NO: of the Sequence Listing. In other embodiments, the nucleic acid is at least 30, 50, 100, 250 or more nucleotides in length. As used herein, the term "hybridizes under stringent conditions" is intended to describe conditions for hybridization and washing under which nucleotide sequences at least 60% homologous to each other typically remain hybridized to each other. Preferably, the conditions are such that sequences at least about 65%, more preferably at least about 70%, and even more preferably at least about 75% or more homologous to each other typically remain hybridized to each other. Such stringent conditions are known to one of ordinary skill in the art and can be found in *Current Protocols in Molecular Biology*, John Wiley & Sons, N.Y. (1989), 6.3.1-6.3.6. A preferred, non-limiting example of stringent hybridization conditions are hybridization in 6X sodium chloride/sodium citrate (SSC) at about 45°C, followed by one or more washes in 0.2 X SSC, 0.1% SDS at 50-65°C. Preferably, an isolated nucleic acid molecule of the invention that hybridizes under stringent conditions to a nucleotide sequence of the invention corresponds to a naturally-occurring nucleic acid molecule. As used herein, a "naturally-occurring" nucleic acid molecule refers to an RNA or DNA molecule having a nucleotide sequence that occurs in nature (e.g., encodes a natural protein). In one embodiment, the nucleic acid encodes a natural *C. glutamicum* MP protein.

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In addition to naturally-occurring variants of the MP sequence that may exist in the population, one of ordinary skill in the art will further appreciate that changes can be introduced by mutation into a nucleotide sequence of the invention, thereby

5 leading to changes in the amino acid sequence of the encoded MP protein, without altering the functional ability of the MP protein. For example, nucleotide substitutions leading to amino acid substitutions at "non-essential" amino acid residues can be made in a nucleotide sequence of the invention. A "non-essential"

10 amino acid residue is a residue that can be altered from the wild-type sequence of one of the MP proteins (e.g., an even-numbered SEQ ID NO: of the Sequence Listing) without altering the activity of said MP protein, whereas an "essential" amino acid residue is required for MP protein activity. Other

15 amino acid residues, however, (e.g., those that are not conserved or only semi-conserved in the domain having MP activity) may not be essential for activity and thus are likely to be amenable to alteration without altering MP activity.

20 Accordingly, another aspect of the invention pertains to nucleic acid molecules encoding MP proteins that contain changes in amino acid residues that are not essential for MP activity. Such MP proteins differ in amino acid sequence from a sequence of an even-numbered SEQ ID NO: of the Sequence Listing yet retain

25 at least one of the MP activities described herein. In one embodiment, the isolated nucleic acid molecule comprises a nucleotide sequence encoding a protein, wherein the protein comprises an amino acid sequence at least about 50% homologous to an amino acid sequence of the invention and is capable of

30 catalyzing an enzymatic reaction in an amino acid, vitamin, cofactor, nutraceutical, nucleotide, nucleoside, or trehalose metabolic pathway, or has one or more activities set forth in Table 1. Preferably, the protein encoded by the nucleic acid molecule is at least about 50-60% homologous to the amino acid

35 sequence of one of the odd-numbered SEQ ID NOs of the Sequence Listing, more preferably at least about 60-70% homologous to one of these sequences, even more preferably at least about 70-80%, 80-90%, 90-95% homologous to one of these sequences, and most preferably at least about 96%, 97%, 98%, or 99% homologous to

40 one of the amino acid sequences of the invention.

To determine the percent homology of two amino acid sequences (e.g., one of the amino acid sequences of the invention and a mutant form thereof) or of two nucleic acids, the sequences

45 are aligned for optimal comparison purposes (e.g., gaps can be introduced in the sequence of one protein or nucleic acid for optimal alignment with the other protein or nucleic acid). The

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amino acid residues or nucleotides at corresponding amino acid positions or nucleotide positions are then compared. When a position in one sequence (e.g., one of the amino acid sequences of the invention) is occupied by the same amino acid residue or
5 nucleotide as the corresponding position in the other sequence (e.g., a mutant form of the amino acid sequence), then the molecules are homologous at that position (i.e., as used herein amino acid or nucleic acid "homology" is equivalent to amino acid or nucleic acid "identity"). The percent homology between the
10 two sequences is a function of the number of identical positions shared by the sequences (i.e., % homology = # of identical positions/total # of positions x 100).

An isolated nucleic acid molecule encoding an MP protein
15 homologous to a protein sequence of the invention (e.g., a sequence of an even-numbered SEQ ID NO: of the Sequence Listing) can be created by introducing one or more nucleotide substitutions, additions or deletions into a nucleotide sequence of the invention such that one or more amino acid substitutions,
20 additions or deletions are introduced into the encoded protein. Mutations can be introduced into one of the nucleotide sequences of the invention by standard techniques, such as site-directed mutagenesis and PCR-mediated mutagenesis. Preferably, conservative amino acid substitutions are made at one or more
25 predicted non-essential amino acid residues. A "conservative amino acid substitution" is one in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art. These families include amino acids
30 with basic side chains (e.g., lysine, arginine, histidine), acidic side chains (e.g., aspartic acid, glutamic acid), uncharged polar side chains (e.g., glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (e.g., alanine, valine, leucine, isoleucine, proline,
35 phenylalanine, methionine, tryptophan), beta-branched side chains (e.g., threonine, valine, isoleucine) and aromatic side chains (e.g., tyrosine, phenylalanine, tryptophan, histidine). Thus, a predicted nonessential amino acid residue in an MP protein is preferably replaced with another amino acid residue from the
40 same side chain family. Alternatively, in another embodiment, mutations can be introduced randomly along all or part of an MP coding sequence, such as by saturation mutagenesis, and the resultant mutants can be screened for an MP activity described herein to identify mutants that retain MP activity. Following
45 mutagenesis of the nucleotide sequence of one of the odd-numbered SEQ ID NOs of the Sequence Listing, the encoded protein can be expressed recombinantly and the activity of the protein can be

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determined using, for example, assays described herein (see Example 8 of the Exemplification).

In addition to the nucleic acid molecules encoding MP proteins
5 described above, another aspect of the invention pertains to isolated nucleic acid molecules which are antisense thereto. An "antisense" nucleic acid comprises a nucleotide sequence which is complementary to a "sense" nucleic acid encoding a protein, e.g., complementary to the coding strand of a double-stranded DNA
10 molecule or complementary to an mRNA sequence. Accordingly, an antisense nucleic acid can hydrogen bond to a sense nucleic acid. The antisense nucleic acid can be complementary to an entire MP coding strand, or to only a portion thereof. In one embodiment, an antisense nucleic acid molecule is antisense to a "coding
15 region" of the coding strand of a nucleotide sequence encoding an MP protein. The term "coding region" refers to the region of the nucleotide sequence comprising codons which are translated into amino acid residues. In another embodiment, the antisense nucleic acid molecule is antisense to a "noncoding region" of the coding
20 strand of a nucleotide sequence encoding MP. The term "noncoding region" refers to 5' and 3' sequences which flank the coding region that are not translated into amino acids (i.e., also referred to as 5' and 3' untranslated regions).

25 Given the coding strand sequences encoding MP disclosed herein (e.g., the sequences set forth as odd-numbered SEQ ID NOs in the Sequence Listing), antisense nucleic acids of the invention can be designed according to the rules of Watson and Crick base pairing. The antisense nucleic acid molecule can be complementary
30 to the entire coding region of MP mRNA, but more preferably is an oligonucleotide which is antisense to only a portion of the coding or noncoding region of MP mRNA. For example, the antisense oligonucleotide can be complementary to the region surrounding the translation start site of MP mRNA. An antisense
35 oligonucleotide can be, for example, about 5, 10, 15, 20, 25, 30, 35, 40, 45 or 50 nucleotides in length. An antisense nucleic acid of the invention can be constructed using chemical synthesis and enzymatic ligation reactions using procedures known in the art. For example, an antisense nucleic acid (e.g., an antisense
40 oligonucleotide) can be chemically synthesized using naturally occurring nucleotides or variously modified nucleotides designed to increase the biological stability of the molecules or to increase the physical stability of the duplex formed between the antisense and sense nucleic acids, e.g., phosphorothioate
45 derivatives and acridine substituted nucleotides can be used. Examples of modified nucleotides which can be used to generate the antisense nucleic acid include 5-fluorouracil, 5-bromouracil,

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- 5-chlorouracil, 5-iodouracil, hypoxanthine, xanthine, 4-acetylcytosine, 5-(carboxyhydroxymethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, 5 beta-D-galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, 10 beta-D-mannosylqueosine, 5'-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5- oxyacetic acid 15 methylester, uracil-5-oxyacetic acid (v), 5-methyl-2-thiouracil, 3-(3-amino-3-N-2-carboxypropyl) uracil, (acp3)w, and 2,6-diaminopurine. Alternatively, the antisense nucleic acid can be produced biologically using an expression vector into which a nucleic acid has been subcloned in an antisense orientation 20 (*i.e.*, RNA transcribed from the inserted nucleic acid will be of an antisense orientation to a target nucleic acid of interest, described further in the following subsection).

- The antisense nucleic acid molecules of the invention are 25 typically administered to a cell or generated *in situ* such that they hybridize with or bind to cellular mRNA and/or genomic DNA encoding an MP protein to thereby inhibit expression of the protein, *e.g.*, by inhibiting transcription and/or translation. The hybridization can be by conventional nucleotide 30 complementarity to form a stable duplex, or, for example, in the case of an antisense nucleic acid molecule which binds to DNA duplexes, through specific interactions in the major groove of the double helix. The antisense molecule can be modified such that it specifically binds to a receptor or an antigen expressed 35 on a selected cell surface, *e.g.*, by linking the antisense nucleic acid molecule to a peptide or an antibody which binds to a cell surface receptor or antigen. The antisense nucleic acid molecule can also be delivered to cells using the vectors described herein. To achieve sufficient intracellular 40 concentrations of the antisense molecules, vector constructs in which the antisense nucleic acid molecule is placed under the control of a strong prokaryotic, viral, or eukaryotic promoter are preferred.
- 45 In yet another embodiment, the antisense nucleic acid molecule of the invention is an α -anomeric nucleic acid molecule. An α -anomeric nucleic acid molecule forms specific double-stranded

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hybrids with complementary RNA in which, contrary to the usual β -units, the strands run parallel to each other (Gaultier et al. (1987) *Nucleic Acids. Res.* 15:6625-6641). The antisense nucleic acid molecule can also comprise a 2'-o-methylribonucleotide
5 (Inoue et al. (1987) *Nucleic Acids Res.* 15:6131-6148) or a chimeric RNA-DNA analogue (Inoue et al. (1987) *FEBS Lett.* 215:327-330).

In still another embodiment, an antisense nucleic acid of the
10 invention is a ribozyme. Ribozymes are catalytic RNA molecules with ribonuclease activity which are capable of cleaving a single-stranded nucleic acid, such as an mRNA, to which they have a complementary region. Thus, ribozymes (e.g., hammerhead ribozymes (described in Haselhoff and Gerlach (1988) *Nature*
15 334:585-591)) can be used to catalytically cleave MP mRNA transcripts to thereby inhibit translation of MP mRNA. A ribozyme having specificity for an MP-encoding nucleic acid can be designed based upon the nucleotide sequence of an MP DNA disclosed herein (i.e., SEQ ID NO: 1 (RXA02229). For example, a
20 derivative of a *Tetrahymena* L-19 IVS RNA can be constructed in which the nucleotide sequence of the active site is complementary to the nucleotide sequence to be cleaved in an MP-encoding mRNA. See, e.g., Cech et al. U.S. Patent No. 4,987,071 and Cech et al. U.S. Patent No. 5,116,742. Alternatively, MP mRNA can be used to
25 select a catalytic RNA having a specific ribonuclease activity from a pool of RNA molecules. See, e.g., Bartel, D. and Szostak, J.W. (1993) *Science* 261:1411-1418.

Alternatively, MP gene expression can be inhibited by targeting
30 nucleotide sequences complementary to the regulatory region of an MP nucleotide sequence (e.g., an MP promoter and/or enhancers) to form triple helical structures that prevent transcription of an MP gene in target cells. See generally, Helene, C. (1991) *Anticancer Drug Des.* 6(6):569-84; Helene, C. et al. (1992) *Ann. N.Y. Acad. Sci.* 660:27-36; and Maher, L.J. (1992) *Bioassays*
35 14(12):807-15.

Another aspect of the invention pertains combinations of gene in the methionine and/or lysine metabolism. Preferred combinations
40 are the combination of metZ with metC, metB (encoding Cystathionine-Synthase), metA (encoding homoserine-O-acetyltransferase), metE (encoding Methionine Synthase), methH (encoding Methionine Synthase, herein designated as SEQ ID No: 1), hom (encoding homoserine dehydrogenase), asd
45 (encoding aspartatesemialdehyd dehydrogenase), ask (encoding aspartokinase) and rxa00657 (table 4).

Table 4

	Nucleic Acid SEQ ID NO	Amino Acid SEQ ID NO	Gene name (identifier)	Function
5				
	5	6	MetZ	Acetylhomoserine sulphydrolase
	7	8	RXA00657	

It may be that all of the genes are expressed in a host strain.

10 But it is also possible that only a part of the mentioned genes is chosen, e.g. metZ and metA, or metZ, metA, metH and hom or any other of the possible combinations. The genes may be altered in their nucleotide and in the corresponding amino acid sequence resulting in derivatives in such a way that their activity is

15 altered under physiological conditions which leads to an increase in productivity and/or yield of a desired fine chemical. One class of such alterations or derivatives is well known for the nucleotide sequence of the ask gene encoding aspartokinase. These alterations lead to removal of feed back inhibition by the amino

20 acids lysine and threonine and subsequently to lysine overproduction. In a preferred embodiment the metH gene or altered forms of the metH gene are used in a Corynebacterium strain in combination with ask, hom, metA and metZ or derivatives of these genes. In another preferred embodiment metH or altered

25 forms of the metH gene are used in a Corynebacterium strain in combination with ask, hom, metA, metZ and metE or derivatives of these genes. In a more preferred embodiment the gene combinations metH or altered forms of the metH gene are combined with ask, hom, metA and metZ or derivatives of these genes, or metH is

30 combined with ask, hom, metA, metZ and metE or derivatives of these genes in a Corynebacterium strain and sulfur sources like sulfates, thiosulfates, sulfites and also more reduced sulfur sources like H₂S and sulfides and derivatives are used in the growth medium. Also sulfur sources like methyl mercaptan,

35 methanesulfonic acid, thioglycolates, thiocyanates, thiourea, sulfur containing amino acids like cysteine and other sulfur containing compounds can be fed. Another aspect of the invention pertains to the use of the above mentioned gene combinations in a Corynebacterium strain which is before or after introduction of

40 the genes mutagenized by radiation or by well known mutagenic chemicals and selected for resistancy against high concentrations of the fine chemical of interest, e.g. lysine or methionine or analogues of the desired fine chemical like the methionine analogues ethionine or methyl methionine or others. In another

45 embodiment the gene combinations mentioned above can be expressed in a Corynebacterium strain having particular gene disruptions. Preferred are gene disruptions that encode proteins that favor

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carbon flux to undesired metabolites. In case methionine is the desired fine chemical the formation of lysine may be unfavorable. In such a case the combination of the above mentioned genes should proceed in a *Corynebacterium* strain bearing a gene

5 disruption of the *lysA* gene (encoding diaminopimelate decarboxylase) or the *ddh* gene (encoding the meso-diaminopimelate dehydrogenase catalysing the conversion of tetrahydropicolinate to meso-diaminopimelate). In a preferred embodiment a favorable combination of the above mentioned genes are all altered in such

10 a way that their gene products are not feed back inhibited by endproducts or metabolites of the biosynthetic pathway leading to the desired fine chemical. In the case that the desired fine chemical is methionine, the gene combinations may be expressed in a strain previously treated with mutagenic agents or radiation

15 and selected for the above mentioned resistancies. Additionally the strain should be grown in a growth medium containing one or more of the above mentioned sulfur sources.

Another aspect of the invention pertains combinations of genes

20 involved in the metabolism of trehalose and the combination of genes involved in the metabolism of trehalose and other mono-, oligo- or polymeric saccharides. Preferred are combinations of the gene for trehalose synthase (herein designated as SEQ ID No: 3) with genes disclosed in table 5.

25

Another aspect of the invention is the combination of the gene for trehalose synthase with genes involved in saccharide import, as e.g. the genes for the PTS system (as disclosed in table 5), other saccharide transport systems or proteins facilitating

30 saccharide efflux from the cell into the surrounding environment.

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TABLE 5: PHOSPHOENOLPYRUVATE: SUGAR PHOSPHOTRANSFERASE SYSTEM

5	<u>Nu-</u> <u>cleotide</u> <u>SEQ</u> <u>ID NO</u>	<u>Amino</u> <u>Acid</u> <u>SEQ</u> <u>ID NO</u>	<u>Identifica-</u> <u>tion Code</u>	<u>Function</u>
				PTS SYSTEM, SUCROSE-SPECIFIC IIBC COMPONENT (EIIBC- SCR) (SUCROSE- PERMEASE IIBC COMPONENT (PHOSPHO- TRANSFERASE ENZYME II, IIBC COMPONENT) (EC 2.7.1.69)
10	9	10	RXS00315	PTS SYSTEM, FRUCTOSE-SPECIFIC IIBC COMPONENT (EC 2.7.1.69)
	11	12	RXN01299	PTS SYSTEM, MANNITOL (CRYPTIC) -SPECIFIC IIA COMPO- NENT (EIIA-(C)MTL) (MANNITOL (CRYPTIC)- PERMEASE IIA COMPONENT) (PHOSPHOTRANSFERASE ENZYME II, A COM- PONENT) (EC 2.7.1.69)
	13	14	RXA00951	PHOSPHOENOLPYRUVATE-PROTEIN PHOSPHOTRANSFE- RASE (EC 2.7.3.9)
15	15	16	RXN01244	PHOSPHOCARRIER PROTEIN HPR
	17	18	RXA01300	PTS SYSTEM, MANNITOL (CRYPTIC) -SPECIFIC IIA COMPO- NENT (EIIA-(C)MTL) (MANNITOL (CRYPTIC)-PERMEASE IIA COMPONENT) (PHOSPHOTRANSFERASE ENZYME II, A COM- PONENT) (EC 2.7.1.69)
20	19	20	RXN03002	Membrane Spanning Protein involved in PTS system
	21	22	RXC00953	Membrane Spanning Protein involved in PTS system
	23	24	RXC03001	PTS SYSTEM, GLUCOSE-SPECIFIC IIBC COMPONENT (EC 2.7.1.69)
	25	26	RXN01943	PTS SYSTEM, BETA-GLUCOSIDES-SPECIFIC IIBC COMPO- NENT (EIIBC-BGL) (BETA-GLUCOSIDES- PERMEASE IIBC COMPONENT) (PHOSPHOTRANSFERASE ENZYME II, IIBC COMPONENT) (EC 2.7.1.69)
25	27	28	RXA01503	

Trehalose

30	<u>Nucleic</u> <u>Acid</u> <u>SEQ ID</u> <u>NO</u>	<u>Amino</u> <u>Acid</u> <u>SEQ ID</u> <u>NO</u>	<u>Identifica-</u> <u>tion Code</u>	<u>Function</u>
	29	30	RXN00351	ALPHA,ALPHA-TREHALOSE-PHOSPHATE SYNTHASE (UDP-FORMING) 56 KD SUBUNIT (EC 2.4.1.15)
35	31	32	RXA00347	TREHALOSE-PHOSPHATASE (EC 3.1.3.12)
	33	34	RXN01239	maltooligosyltrehalose synthase
	35	36	RXA02645	maltooligosyltrehalose trehalohydrolase
	37	38	RXN02355	TREHALOSE/MALTOSE BINDING PROTEIN
	39	40	RXN02909	Hypothetical Trehalose-Binding Protein
40	41	42	RXS00349	Hypothetical Trehalose Transport Protein
	43	44	RXS03183	TREHALOSE/MALTOSE BINDING PROTEIN
	45	46	RXC00874	transmembrane protein involved in trehalose metabolism

Another aspect of the invention pertains to the use of the above
 45 mentioned gene combinations in a Corynebacterium strain which
 is before or after introduction of the genes mutagenized by
 radiation or by well known mutagenic chemicals and selected for

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resistancy against high concentrations of feedstock (as e.g. glucose or other saccharides) or the fine chemical of interest, e.g. trehalose or other saccharides.

- 5 In another embodiment the gene combinations mentioned above can be expressed in a *Corynebacterium* strain having particular gene disruptions or gene attenuations (i.e. genes which biological activity is reduced compared to the normal level). Preferred are disruptions or attenuations of genes that encode proteins that
- 10 favor carbon flux to metabolic pathways which do not lead to the desired fine chemical. In case of trehalose being the desired fine chemical, such less desired metabolic pathways may be e.g. glycolysis or pentose phosphate cycle.

15 B. Recombinant Expression Vectors and Host Cells

- Another aspect of the invention pertains to vectors, preferably expression vectors, containing a nucleic acid encoding an MP protein (or a portion thereof) or combinations of genes wherein
- 20 at least one gene encodes for an MP protein. As used herein, the term "vector" refers to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. One type of vector is a "plasmid", which refers to a circular double stranded DNA loop into which additional DNA segments can
- 25 be ligated. Another type of vector is a viral vector, wherein additional DNA segments can be ligated into the viral genome. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (e.g., bacterial vectors having a bacterial origin of replication and episomal mammalian
- 30 vectors). Other vectors (e.g., non-episomal mammalian vectors) are integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. Moreover, certain vectors are capable of directing the expression of genes to which they are operatively linked.
- 35 Such vectors are referred to herein as "expression vectors". In general, expression vectors of utility in recombinant DNA techniques are often in the form of plasmids. In the present specification, "plasmid" and "vector" can be used interchangeably as the plasmid is the most commonly used form of vector. However,
- 40 the invention is intended to include such other forms of expression vectors, such as viral vectors (e.g., replication defective retroviruses, adenoviruses and adeno-associated viruses), which serve equivalent functions.
- 45 The recombinant expression vectors of the invention comprise a nucleic acid of the invention in a form suitable for expression of the nucleic acid in a host cell, which means that the

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recombinant expression vectors include one or more regulatory sequences, selected on the basis of the host cells to be used for expression, which is operatively linked to the nucleic acid sequence to be expressed. Within a recombinant expression vector, 5 "operably linked" is intended to mean that the nucleotide sequence of interest is linked to the regulatory sequence(s) in a manner which allows for expression of the nucleotide sequence (e.g., in an *in vitro* transcription/translation system or in a host cell when the vector is introduced into the host cell). The 10 term "regulatory sequence" is intended to include promoters, repressor binding sites, activator binding sites, enhancers and other expression control elements (e.g., terminators, polyadenylation signals, or other elements of mRNA secondary structure). Such regulatory sequences are described, for example, 15 in Goeddel; *Gene Expression Technology: Methods in Enzymology* 185, Academic Press, San Diego, CA (1990). Regulatory sequences include those which direct constitutive expression of a nucleotide sequence in many types of host cell and those which direct expression of the nucleotide sequence only in certain host 20 cells. Preferred regulatory sequences are, for example, promoters such as *cos*-, *tac*-, *trp*-, *tet*-, *trp-tet*-, *lpp*-, *lac*-, *lpp-lac*-, *lacIq*-, *T7*-, *T5*-, *T3*-, *gal*-, *trc*-, *ara*-, *SP6*-, *arny*, *SPO2*, $\bar{\epsilon}$ -*P_R*- or $\bar{\epsilon}$ *P_L*, which are used preferably in bacteria. Additional regulatory sequences are, for example, promoters from yeasts and 25 fungi, such as *ADC1*, *MFá*, *AC*, *P-60*, *CYC1*, *GAPDH*, *TEF*, *rp28*, *ADH*, promoters from plants such as *CaMV/35S*, *SSU*, *OCS*, *lib4*, *usp*, *STLS1*, *B33*, *nos* or *ubiquitin*- or *phaseolin*-promoters. It is also possible to use artificial promoters. It will be appreciated by one of ordinary skill in the art that the design of the 30 expression vector can depend on such factors as the choice of the host cell to be transformed, the level of expression of protein desired, etc. The expression vectors of the invention can be introduced into host cells to thereby produce proteins or peptides, including fusion proteins or peptides, encoded by 35 nucleic acids as described herein (e.g., MP proteins, mutant forms of MP proteins, fusion proteins, etc.).

The recombinant expression vectors of the invention can be designed for expression of MP proteins in prokaryotic or 40 eukaryotic cells. For example, MP genes can be expressed in bacterial cells such as *C. glutamicum*, insect cells (using baculovirus expression vectors), yeast and other fungal cells (see Romanos, M.A. et al. (1992) "Foreign gene expression in yeast: a review", *Yeast* 8: 423-488; van den Hondel, C.A.M.J.J. et 45 al. (1991) "Heterologous gene expression in filamentous fungi" in: *More Gene Manipulations in Fungi*, J.W. Bennet & L.L. Lasure, eds., p. 396-428: Academic Press: San Diego; and van den Hondel,

- C.A.M.J.J. & Punt, P.J. (1991) "Gene transfer systems and vector development for filamentous fungi, in: Applied Molecular Genetics of Fungi, Peberdy, J.F. et al., eds., p. 1-28, Cambridge University Press: Cambridge), algae and multicellular plant
- 5 cells (see Schmidt, R. and Willmitzer, L. (1988) High efficiency *Agrobacterium tumefaciens* -mediated transformation of *Arabidopsis thaliana* leaf and cotyledon explants" *Plant Cell Rep.*: 583-586), or mammalian cells. Suitable host cells are discussed further in Goeddel, *Gene Expression Technology: Methods in Enzymology* 185,
- 10 Academic Press, San Diego, CA (1990). Alternatively, the recombinant expression vector can be transcribed and translated *in vitro*, for example using T7 promoter regulatory sequences and T7 polymerase.
- 15 Expression of proteins in prokaryotes is most often carried out with vectors containing constitutive or inducible promoters directing the expression of either fusion or non-fusion proteins. Fusion vectors add a number of amino acids to a protein encoded therein, usually to the amino terminus of the recombinant protein
- 20 but also to the C-terminus or fused within suitable regions in the proteins. Such fusion vectors typically serve three purposes: 1) to increase expression of recombinant protein; 2) to increase the solubility of the recombinant protein; and 3) to aid in the purification of the recombinant protein by acting as a ligand in
- 25 affinity purification. Often, in fusion expression vectors, a proteolytic cleavage site is introduced at the junction of the fusion moiety and the recombinant protein to enable separation of the recombinant protein from the fusion moiety subsequent to purification of the fusion protein. Such enzymes, and their
- 30 cognate recognition sequences, include Factor Xa, thrombin and enterokinase.

Typical fusion expression vectors include pGEX (Pharmacia Biotech Inc; Smith, D.B. and Johnson, K.S. (1988) *Gene* 67:31-40), pMAL

35 (New England Biolabs, Beverly, MA) and pRIT5 (Pharmacia, Piscataway, NJ) which fuse glutathione S-transferase (GST), maltose E binding protein, or protein A, respectively, to the target recombinant protein. In one embodiment, the coding sequence of the MP protein is cloned into a pGEX expression

40 vector to create a vector encoding a fusion protein comprising, from the N-terminus to the C-terminus, GST-thrombin cleavage site-X protein. The fusion protein can be purified by affinity chromatography using glutathione-agarose resin. Recombinant MP protein unfused to GST can be recovered by cleavage of the fusion

45 protein with thrombin.

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- Examples of suitable inducible non-fusion *E. coli* expression vectors include pTrc (Amann et al., (1988) *Gene* 69:301-315) pLG338, pACYC184, pBR322, pUC18, pUC19, pKC30, pRep4, pHS1, pHS2, pPLc236, pMBL24, pLG200, pUR290, pIN-III113-B1, *egt11*, pBdCl, and
- 5 pET 11d (Studier et al., *Gene Expression Technology: Methods in Enzymology* 185, Academic Press, San Diego, California (1990) 60-89; and Pouwels et al., eds. (1985) *Cloning Vectors*. Elsevier: New York ISBN 0 444 904018). Target gene expression from the pTrc vector relies on host RNA polymerase transcription from a hybrid
- 10 *trp-lac* fusion promoter. Target gene expression from the pET 11d vector relies on transcription from a T7 *gn10-lac* fusion promoter mediated by a coexpressed viral RNA polymerase (T7 *gn1*). This viral polymerase is supplied by host strains BL21(DE3) or HMS174(DE3) from a resident λ prophage harboring a T7 *gn1* gene
- 15 under the transcriptional control of the *lacUV 5* promoter. For transformation of other varieties of bacteria, appropriate vectors may be selected. For example, the plasmids pIJ101, pIJ364, pIJ702 and pIJ361 are known to be useful in transforming *Streptomyces*, while plasmids pUB110, pC194, or pBD214 are suited
- 20 for transformation of *Bacillus* species. Several plasmids of use in the transfer of genetic information into *Corynebacterium* include pHM1519, pBL1, pSA77, or pAJ667 (Pouwels et al., eds. (1985) *Cloning Vectors*. Elsevier: New York ISBN 0 444 904018).
- 25 One strategy to maximize recombinant protein expression is to express the protein in a host bacteria with an impaired capacity to proteolytically cleave the recombinant protein (Gottesman, S., *Gene Expression Technology: Methods in Enzymology* 185, Academic Press, San Diego, California (1990) 119-128). Another strategy is
- 30 to alter the nucleic acid sequence of the nucleic acid to be inserted into an expression vector so that the individual codons for each amino acid are those preferentially utilized in the bacterium chosen for expression, such as *C. glutamicum* (Wada et al. (1992) *Nucleic Acids Res.* 20:2111-2118). Such alteration of
- 35 nucleic acid sequences of the invention can be carried out by standard DNA synthesis techniques.

- In another embodiment, the MP protein expression vector is a yeast expression vector. Examples of vectors for expression in
- 40 yeast *S. cerevisiae* include pYepSec1 (Baldari, et al., (1987) *Embo J.* 6:229-234), , 2 i, pAG-1, Yep6, Yep13, pEMBLye23, pMFa (Kurjan and Herskowitz, (1982) *Cell* 30:933-943), pJRY88 (Schultz et al., (1987) *Gene* 54:113-123), and pYES2 (Invitrogen Corporation, San Diego, CA). Vectors and methods for the
- 45 construction of vectors appropriate for use in other fungi, such as the filamentous fungi, include those detailed in: van den Hondel, C.A.M.J.J. & Punt, P.J. (1991) "Gene transfer systems and

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vector development for filamentous fungi, in: *Applied Molecular Genetics of Fungi*, J.F. Peberdy, et al., eds., p. 1-28, Cambridge University Press: Cambridge, and Pouwels et al., eds. (1985) *Cloning Vectors*. Elsevier: New York (ISBN 0 444 904018).

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Alternatively, the MP proteins of the invention can be expressed in insect cells using baculovirus expression vectors. Baculovirus vectors available for expression of proteins in cultured insect cells (e.g., Sf 9 cells) include the pAc series (Smith et al.

- 10 (1983) *Mol. Cell Biol.* 3:2156-2165) and the pVL series (Lucklow and Summers (1989) *Virology* 170:31-39).

In another embodiment, the MP proteins of the invention may be expressed in unicellular plant cells (such as algae) or in plant
15 cells from higher plants (e.g., the spermatophytes, such as crop plants). Examples of plant expression vectors include those detailed in: Becker, D., Kemper, E., Schell, J. and Masterson, R. (1992) "New plant binary vectors with selectable markers located proximal to the left border", *Plant Mol. Biol.* 20: 1195-1197;
20 and Bevan, M.W. (1984) "Binary *Agrobacterium* vectors for plant transformation", *Nucl. Acid. Res.* 12: 8711-8721, and include pLGV23, pGHIac+, pBIN19, pAK2004, and pDH51 (Pouwels et al., eds. (1985) *Cloning Vectors*. Elsevier: New York ISBN 0 444 904018).

- 25 In yet another embodiment, a nucleic acid of the invention is expressed in mammalian cells using a mammalian expression vector. Examples of mammalian expression vectors include pCDM8 (Seed, B. (1987) *Nature* 329:840) and pMT2PC (Kaufman et al. (1987) *EMBO J.* 6:187-195). When used in mammalian cells, the expression vector's
30 control functions are often provided by viral regulatory elements. For example, commonly used promoters are derived from polyoma, Adenovirus 2, cytomegalovirus and Simian Virus 40. For other suitable expression systems for both prokaryotic and eukaryotic cells see chapters 16 and 17 of Sambrook, J., Fritsh,
35 E. F., and Maniatis, T. *Molecular Cloning: A Laboratory Manual*. 2nd, ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989.

In another embodiment, the recombinant mammalian expression
40 vector is capable of directing expression of the nucleic acid preferentially in a particular cell type (e.g., tissue-specific regulatory elements are used to express the nucleic acid). Tissue-specific regulatory elements are known in the art. Non-limiting examples of suitable tissue-specific promoters
45 include the albumin promoter (liver-specific; Pinkert et al. (1987) *Genes Dev.* 1:268-277), lymphoid-specific promoters (Calame and Eaton (1988) *Adv. Immunol.* 43:235-275), in particular

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promoters of T cell receptors (Winoto and Baltimore (1989) *EMBO J.* 8:729-733) and immunoglobulins (Banerji et al. (1983) *Cell* 33:729-740; Queen and Baltimore (1983) *Cell* 33:741-748), neuron-specific promoters (e.g., the neurofilament promoter; 5 Byrne and Ruddle (1989) *PNAS* 86:5473-5477), pancreas-specific promoters (Edlund et al. (1985) *Science* 230:912-916), and mammary gland-specific promoters (e.g., milk whey promoter; U.S. Patent No. 4,873,316 and European Application Publication No. 264,166). Developmentally-regulated promoters are also encompassed, for 10 example the murine hox promoters (Kessel and Gruss (1990) *Science* 249:374-379) and the α -fetoprotein promoter (Campes and Tilghman (1989) *Genes Dev.* 3:537-546).

The invention further provides a recombinant expression vector 15 comprising a DNA molecule of the invention cloned into the expression vector in an antisense orientation. That is, the DNA molecule is operatively linked to a regulatory sequence in a manner which allows for expression (by transcription of the DNA molecule) of an RNA molecule which is antisense to MP mRNA. 20 Regulatory sequences operatively linked to a nucleic acid cloned in the antisense orientation can be chosen which direct the continuous expression of the antisense RNA molecule in a variety of cell types, for instance viral promoters and/or enhancers, or regulatory sequences can be chosen which direct constitutive, 25 tissue specific or cell type specific expression of antisense RNA. The antisense expression vector can be in the form of a recombinant plasmid, phagemid or attenuated virus in which antisense nucleic acids are produced under the control of a high efficiency regulatory region, the activity of which can be 30 determined by the cell type into which the vector is introduced. For a discussion of the regulation of gene expression using antisense genes see Weintraub, H. et al., *Antisense RNA as a molecular tool for genetic analysis, Reviews - Trends in Genetics*, Vol. 1(1) 1986.

35 Another aspect of the invention pertains to host cells into which a recombinant expression vector of the invention has been introduced. The terms "host cell" and "recombinant host cell" are used interchangeably herein. It is understood that such 40 terms refer not only to the particular subject cell but to the progeny or potential progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included 45 within the scope of the term as used herein.

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A host cell can be any prokaryotic or eukaryotic cell. For example, an MP protein can be expressed in bacterial cells such as *C. glutamicum*, insect cells, yeast or mammalian cells (such as Chinese hamster ovary cells (CHO) or COS cells). Other suitable
5 host cells are known to those of ordinary skill in the art.

Microorganisms related to *Corynebacterium glutamicum* which may be conveniently used as host cells for the nucleic acid and protein molecules of the invention are set forth in Table 2.

- 10 Vector DNA can be introduced into prokaryotic or eukaryotic cells via conventional transformation or transfection techniques. As used herein, the terms "transformation" and "transfection", "conjugation" and "transduction" are intended to refer to a variety of art-recognized techniques for introducing foreign
15 nucleic acid (e.g., linear DNA or RNA (e.g., a linearized vector or a gene construct alone without a vector) or nucleic acid in the form of a vector (e.g., a plasmid, phage, phasmid, phagemid, transposon or other DNA) into a host cell, including calcium phosphate or calcium chloride co-precipitation,
20 DEAE-dextran-mediated transfection, lipofection, natural competence, chemical-mediated transfer, or electroporation. Suitable methods for transforming or transfecting host cells can be found in Sambrook, et al. (*Molecular Cloning: A Laboratory Manual*. 2nd, ed., Cold Spring Harbor Laboratory, Cold Spring
25 Harbor Laboratory Press, Cold Spring Harbor, NY, 1989), and other laboratory manuals.

- For stable transfection of mammalian cells, it is known that, depending upon the expression vector and transfection technique
30 used, only a small fraction of cells may integrate the foreign DNA into their genome. In order to identify and select these integrants, a gene that encodes a selectable marker (e.g., resistance to antibiotics) is generally introduced into the host cells along with the gene of interest. Preferred selectable
35 markers include those which confer resistance to drugs, such as G418, hygromycin and methotrexate. Nucleic acid encoding a selectable marker can be introduced into a host cell on the same vector as that encoding an MP protein or can be introduced on a separate vector. Cells stably transfected with the introduced
40 nucleic acid can be identified by drug selection (e.g., cells that have incorporated the selectable marker gene will survive, while the other cells die).

- To create a homologous recombinant microorganism, a vector is
45 prepared which contains at least a portion of an MP gene into which a deletion, addition or substitution has been introduced to thereby alter, e.g., functionally disrupt, the MP gene.

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Preferably, this MP gene is a *Corynebacterium glutamicum* MP gene, but it can be a homologue from a related bacterium or even from a mammalian, yeast, or insect source. In a preferred embodiment, the vector is designed such that, upon homologous recombination, the endogenous MP gene is functionally disrupted (i.e., no longer encodes a functional protein; also referred to as a "knock out" vector). Alternatively, the vector can be designed such that, upon homologous recombination, the endogenous MP gene is mutated or otherwise altered but still encodes functional protein (e.g., the upstream regulatory region can be altered to thereby alter the expression of the endogenous MP protein). In the homologous recombination vector, the altered portion of the MP gene is flanked at its 5' and 3' ends by additional nucleic acid of the MP gene to allow for homologous recombination to occur between the exogenous MP gene carried by the vector and an endogenous MP gene in a microorganism. The additional flanking MP nucleic acid is of sufficient length for successful homologous recombination with the endogenous gene. Typically, several kilobases of flanking DNA (both at the 5' and 3' ends) are included in the vector (see e.g., Thomas, K.R., and Capecchi, M.R. (1987) Cell 51: 503 for a description of homologous recombination vectors). The vector is introduced into a microorganism (e.g., by electroporation) and cells in which the introduced MP gene has homologously recombined with the endogenous MP gene are selected, using art-known techniques.

In another embodiment, recombinant microorganisms can be produced which contain selected systems which allow for regulated expression of the introduced gene. For example, inclusion of an MP gene on a vector placing it under control of the lac operon permits expression of the MP gene only in the presence of IPTG. Such regulatory systems are well known in the art.

In another embodiment, an endogenous MP gene in a host cell is disrupted (e.g., by homologous recombination or other genetic means known in the art) such that expression of its protein product does not occur. In another embodiment, an endogenous or introduced MP gene in a host cell has been altered by one or more point mutations, deletions, or inversions, but still encodes a functional MP protein. In still another embodiment, one or more of the regulatory regions (e.g., a promoter, repressor, or inducer) of an MP gene in a microorganism has been altered (e.g., by deletion, truncation, inversion, or point mutation) such that the expression of the MP gene is modulated. One of ordinary skill in the art will appreciate that host cells containing more than one of the described MP gene and protein modifications may be

readily produced using the methods of the invention, and are meant to be included in the present invention.

A host cell of the invention, such as a prokaryotic or eukaryotic host cell in culture, can be used to produce (*i.e.*, express) an MP protein. Accordingly, the invention further provides methods for producing MP proteins using the host cells of the invention. In one embodiment, the method comprises culturing the host cell of invention (into which a recombinant expression vector encoding an MP protein has been introduced, or into which genome has been introduced a gene encoding a wild-type or altered MP protein) in a suitable medium until MP protein is produced. In another embodiment, the method further comprises isolating MP proteins from the medium or the host cell.

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C. Isolated MP Proteins

Another aspect of the invention pertains to isolated MP proteins, and biologically active portions thereof. An "isolated" or "purified" protein or biologically active portion thereof is substantially free of cellular material when produced by recombinant DNA techniques, or chemical precursors or other chemicals when chemically synthesized. The language "substantially free of cellular material" includes preparations of MP protein in which the protein is separated from cellular components of the cells in which it is naturally or recombinantly produced. In one embodiment, the language "substantially free of cellular material" includes preparations of MP protein having less than about 30% (by dry weight) of non-MP protein (also referred to herein as a "contaminating protein"), more preferably less than about 20% of non-MP protein, still more preferably less than about 10% of non-MP protein, and most preferably less than about 5% non-MP protein. When the MP protein or biologically active portion thereof is recombinantly produced, it is also preferably substantially free of culture medium, *i.e.*, culture medium represents less than about 20%, more preferably less than about 10%, and most preferably less than about 5% of the volume of the protein preparation. The language "substantially free of chemical precursors or other chemicals" includes preparations of MP protein in which the protein is separated from chemical precursors or other chemicals which are involved in the synthesis of the protein. In one embodiment, the language "substantially free of chemical precursors or other chemicals" includes preparations of MP protein having less than about 30% (by dry weight) of chemical precursors or non-MP chemicals, more preferably less than about 20% chemical precursors or non-MP chemicals, still more preferably less than about 10% chemical

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precursors or non-MP chemicals, and most preferably less than about 5% chemical precursors or non-MP chemicals. In preferred embodiments, isolated proteins or biologically active portions thereof lack contaminating proteins from the same organism
5 from which the MP protein is derived. Typically, such proteins are produced by recombinant expression of, for example, a *C. glutamicum* MP protein in a microorganism such as *C. glutamicum*.

An isolated MP protein or a portion thereof of the invention
10 can catalyze an enzymatic reaction in an amino acid, vitamin, cofactor, nutraceutical, nucleotide, nucleoside, or trehalose metabolic pathway, or has one or more of the activities set forth in Table 1. In preferred embodiments, the protein or portion thereof comprises an amino acid sequence which is sufficiently
15 homologous to an amino acid sequence of the invention (e.g., a sequence of an even-numbered SEQ ID NO: of the Sequence Listing) such that the protein or portion thereof maintains the ability to catalyze an enzymatic reaction in an amino acid, vitamin, cofactor, nutraceutical, nucleotide, nucleoside, or trehalose
20 metabolic pathway. The portion of the protein is preferably a biologically active portion as described herein. In another preferred embodiment, an MP protein of the invention has an amino acid sequence set forth as an even-numbered SEQ ID NO: of the Sequence Listing. In yet another preferred embodiment,
25 the MP protein has an amino acid sequence which is encoded by a nucleotide sequence which hybridizes, e.g., hybridizes under stringent conditions, to a nucleotide sequence of the invention (e.g., a sequence of an odd-numbered SEQ ID NO: of the Sequence Listing). In still another preferred embodiment, the MP protein
30 has an amino acid sequence which is encoded by a nucleotide sequence that is preferably at least about 63%, 64%, 65%, 66%, 67%, 68%, 69%, or 70%, more preferably at least about 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, or 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, or 90%, or 91%, 92%, 93%, 94%, and even
35 more preferably at least about 95%, 96%, 97%, 98%, 99% or more homologous to one of the nucleic acid sequences of the invention, or a portion thereof. Ranges and identity values intermediate to the above-recited values, (e.g., 70-90% identical or 80-95% identical) are also intended to be encompassed by the present
40 invention. For example, ranges of identity values using a combination of any of the above values recited as upper and/or lower limits are intended to be included. The preferred MP proteins of the present invention also preferably possess at least one of the MP activities described herein. For example, a
45 preferred MP protein of the present invention includes an amino acid sequence encoded by a nucleotide sequence which hybridizes, e.g., hybridizes under stringent conditions, to a nucleotide

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sequence of the invention, and which can catalyze an enzymatic reaction in an amino acid, vitamin, cofactor, nutraceutical, nucleotide, nucleoside, or trehalose metabolic pathway, or which has one or more of the activities set forth in Table 1.

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In other embodiments, the MP protein is substantially homologous to an amino acid sequence of the invention (e.g., a sequence of an even-numbered SEQ ID NO: of the Sequence Listing) and retains the functional activity of the protein of one of the amino acid sequences of the invention yet differs in amino acid sequence due to natural variation or mutagenesis, as described in detail in subsection I above. Accordingly, in another embodiment, the MP protein is a protein which comprises an amino acid sequence which is preferably at least about 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, more preferably at least about 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, or 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, or 90%, or 91%, 92%, 93%, 94%, and even more preferably at least about 95%, 96%, 97%, 98%, 99% or more homologous to an entire amino acid sequence of the invention and which has at least one of the MP activities described herein. Ranges and identity values intermediate to the above-recited values, (e.g., 70-90% identical or 80-95% identical) are also intended to be encompassed by the present invention. For example, ranges of identity values using a combination of any of the above values recited as upper and/or lower limits are intended to be included. In another embodiment, the invention pertains to a full length *C. glutamicum* protein which is substantially homologous to an entire amino acid sequence of the invention.

Biologically active portions of an MP protein include peptides comprising amino acid sequences derived from the amino acid sequence of an MP protein, e.g., an amino acid sequence of an even-numbered SEQ ID NO: of the Sequence Listing or the amino acid sequence of a protein homologous to an MP protein, which include fewer amino acids than a full length MP protein or the full length protein which is homologous to an MP protein, and exhibit at least one activity of an MP protein. Typically, biologically active portions (peptides, e.g., peptides which are, for example, 5, 10, 15, 20, 30, 35, 36, 37, 38, 39, 40, 50, 100 or more amino acids in length) comprise a domain or motif with at least one activity of an MP protein. Moreover, other biologically active portions, in which other regions of the protein are deleted, can be prepared by recombinant techniques and evaluated for one or more of the activities described herein. Preferably, the biologically active portions of an MP protein include one

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or more selected domains/motifs or portions thereof having biological activity.

MP proteins are preferably produced by recombinant DNA techniques. For example, a nucleic acid molecule encoding the protein is cloned into an expression vector (as described above), the expression vector is introduced into a host cell (as described above) and the MP protein is expressed in the host cell. The MP protein can then be isolated from the cells by an appropriate purification scheme using standard protein purification techniques. Alternative to recombinant expression, an MP protein, polypeptide, or peptide can be synthesized chemically using standard peptide synthesis techniques. Moreover, native MP protein can be isolated from cells (e.g., endothelial cells), for example using an anti-MP antibody, which can be produced by standard techniques utilizing an MP protein or fragment thereof of this invention.

The invention also provides MP chimeric or fusion proteins. As used herein, an MP "chimeric protein" or "fusion protein" comprises an MP polypeptide operatively linked to a non-MP polypeptide. An "MP polypeptide" refers to a polypeptide having an amino acid sequence corresponding to MP, whereas a "non-MP polypeptide" refers to a polypeptide having an amino acid sequence corresponding to a protein which is not substantially homologous to the MP protein, e.g., a protein which is different from the MP protein and which is derived from the same or a different organism. Within the fusion protein, the term "operatively linked" is intended to indicate that the MP polypeptide and the non-MP polypeptide are fused in-frame to each other. The non-MP polypeptide can be fused to the N-terminus or C-terminus of the MP polypeptide. For example, in one embodiment the fusion protein is a GST-MP fusion protein in which the MP sequences are fused to the C-terminus of the GST sequences. Such fusion proteins can facilitate the purification of recombinant MP proteins. In another embodiment, the fusion protein is an MP protein containing a heterologous signal sequence at its N-terminus. In certain host cells (e.g., mammalian host cells), expression and/or secretion of an MP protein can be increased through use of a heterologous signal sequence.

Preferably, an MP chimeric or fusion protein of the invention is produced by standard recombinant DNA techniques. For example, DNA fragments coding for the different polypeptide sequences are ligated together in-frame in accordance with conventional techniques, for example by employing blunt-ended or stagger-ended termini for ligation, restriction enzyme digestion to provide for

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appropriate termini, filling-in of cohesive ends as appropriate, alkaline phosphatase treatment to avoid undesirable joining, and enzymatic ligation. In another embodiment, the fusion gene can be synthesized by conventional techniques including automated DNA
5 synthesizers. Alternatively, PCR amplification of gene fragments can be carried out using anchor primers which give rise to complementary overhangs between two consecutive gene fragments which can subsequently be annealed and reamplified to generate a chimeric gene sequence (see, for example, *Current Protocols in*
10 *Molecular Biology*, eds. Ausubel et al. John Wiley & Sons: 1992). Moreover, many expression vectors are commercially available that already encode a fusion moiety (e.g., a GST polypeptide). An MP-encoding nucleic acid can be cloned into such an expression vector such that the fusion moiety is linked in-frame to the MP
15 protein.

Homologues of the MP protein can be generated by mutagenesis, e.g., discrete point mutation or truncation of the MP protein. As used herein, the term "homologue" refers to a variant form of the
20 MP protein which acts as an agonist or antagonist of the activity of the MP protein. An agonist of the MP protein can retain substantially the same, or a subset, of the biological activities of the MP protein. An antagonist of the MP protein can inhibit one or more of the activities of the naturally occurring form of
25 the MP protein, by, for example, competitively binding to a downstream or upstream member of the MP cascade which includes the MP protein. Thus, the *C. glutamicum* MP protein and homologues thereof of the present invention may modulate the activity of one or more metabolic pathways in which MP proteins play a role in
30 this microorganism.

In an alternative embodiment, homologues of the MP protein can be identified by screening combinatorial libraries of mutants, e.g., truncation mutants, of the MP protein for MP protein agonist or
35 antagonist activity. In one embodiment, a variegated library of MP variants is generated by combinatorial mutagenesis at the nucleic acid level and is encoded by a variegated gene library. A variegated library of MP variants can be produced by, for example, enzymatically ligating a mixture of synthetic
40 oligonucleotides into gene sequences such that a degenerate set of potential MP sequences is expressible as individual polypeptides, or alternatively, as a set of larger fusion proteins (e.g., for phage display) containing the set of MP sequences therein. There are a variety of methods which can
45 be used to produce libraries of potential MP homologues from a degenerate oligonucleotide sequence. Chemical synthesis of a degenerate gene sequence can be performed in an automatic

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- DNA synthesizer, and the synthetic gene then ligated into an appropriate expression vector. Use of a degenerate set of genes allows for the provision, in one mixture, of all of the sequences encoding the desired set of potential MP sequences. Methods for synthesizing degenerate oligonucleotides are known in the art (see, e.g., Narang, S.A. (1983) *Tetrahedron* 39:3; Itakura et al. (1984) *Annu. Rev. Biochem.* 53:323; Itakura et al. (1984) *Science* 198:1056; Ike et al. (1983) *Nucleic Acid Res.* 11:477.
- 10 In addition, libraries of fragments of the MP protein coding can be used to generate a variegated population of MP fragments for screening and subsequent selection of homologues of an MP protein. In one embodiment, a library of coding sequence fragments can be generated by treating a double stranded
- 15 PCR fragment of an MP coding sequence with a nuclease under conditions wherein nicking occurs only about once per molecule, denaturing the double stranded DNA, renaturing the DNA to form double stranded DNA which can include sense/antisense pairs from different nicked products, removing single stranded portions from
- 20 reformed duplexes by treatment with S1 nuclease, and ligating the resulting fragment library into an expression vector. By this method, an expression library can be derived which encodes N-terminal, C-terminal and internal fragments of various sizes of the MP protein.
- 25 Several techniques are known in the art for screening gene products of combinatorial libraries made by point mutations or truncation, and for screening cDNA libraries for gene products having a selected property. Such techniques are adaptable
- 30 for rapid screening of the gene libraries generated by the combinatorial mutagenesis of MP homologues. The most widely used techniques, which are amenable to high through-put analysis, for screening large gene libraries typically include cloning the gene library into replicable expression vectors, transforming
- 35 appropriate cells with the resulting library of vectors, and expressing the combinatorial genes under conditions in which detection of a desired activity facilitates isolation of the vector encoding the gene whose product was detected. Recursive ensemble mutagenesis (REM), a new technique which enhances the
- 40 frequency of functional mutants in the libraries, can be used in combination with the screening assays to identify MP homologues (Arkin and Yourvan (1992) *PNAS* 89:7811-7815; Delgrave et al. (1993) *Protein Engineering* 6(3):327-331).

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In another embodiment, cell based assays can be exploited to analyze a variegated MP library, using methods well known in the art.

5 D. Uses and Methods of the Invention

The nucleic acid molecules, proteins, protein homologues, fusion proteins, primers, vectors, and host cells described herein can be used in one or more of the following methods: identification of *C. glutamicum* and related organisms; mapping of genomes of organisms related to *C. glutamicum*; identification and localization of *C. glutamicum* sequences of interest; evolutionary studies; determination of MP protein regions required for function; modulation of an MP protein activity; modulation of the activity of an MP pathway; and modulation of cellular production of a desired compound, such as a fine chemical. The MP nucleic acid molecules of the invention have a variety of uses. First, they may be used to identify an organism as being *Corynebacterium glutamicum* or a close relative thereof. Also, they may be used to identify the presence of *C. glutamicum* or a relative thereof in a mixed population of microorganisms. The invention provides the nucleic acid sequences of a number of *C. glutamicum* genes; by probing the extracted genomic DNA of a culture of a unique or mixed population of microorganisms under stringent conditions with a probe spanning a region of a *C. glutamicum* gene which is unique to this organism, one can ascertain whether this organism is present. Although *Corynebacterium glutamicum* itself is not pathogenic to humans, it is related to species which are human pathogens, such as *Corynebacterium diphtheriae*. *Corynebacterium diphtheriae* is the causative agent of diphtheria, a rapidly developing, acute, febrile infection which involves both local and systemic pathology. In this disease, a local lesion develops in the upper respiratory tract and involves necrotic injury to epithelial cells; the bacilli secrete toxin which is disseminated through this lesion to distal susceptible tissues of the body. Degenerative changes brought about by the inhibition of protein synthesis in these tissues, which include heart, muscle, peripheral nerves, adrenals, kidneys, liver and spleen, result in the systemic pathology of the disease. Diphtheria continues to have high incidence in many parts of the world, including Africa, Asia, Eastern Europe and the independent states of the former Soviet Union. An ongoing epidemic of diphtheria in the latter two regions has resulted in at least 5,000 deaths since 1990.

In one embodiment, the invention provides a method of identifying the presence or activity of *Corynebacterium diphtheriae* in a subject. This method includes detection of one or more of the

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nucleic acid or amino acid sequences of the invention (e.g., the sequences set forth as odd-numbered or even-numbered SEQ ID NOs, respectively, in the Sequence Listing) in a subject, thereby detecting the presence or activity of *Corynebacterium diphtheriae* in the subject. *C. glutamicum* and *C. diphtheriae* are related bacteria, and many of the nucleic acid and protein molecules in *C. glutamicum* are homologous to *C. diphtheriae* nucleic acid and protein molecules, and can therefore be used to detect *C. diphtheriae* in a subject.

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The nucleic acid and protein molecules of the invention may also serve as markers for specific regions of the genome. This has utility not only in the mapping of the genome, but also for functional studies of *C. glutamicum* proteins. For example, to identify the region of the genome to which a particular *C. glutamicum* DNA-binding protein binds, the *C. glutamicum* genome could be digested, and the fragments incubated with the DNA-binding protein. Those which bind the protein may be additionally probed with the nucleic acid molecules of the invention, preferably with readily detectable labels; binding of such a nucleic acid molecule to the genome fragment enables the localization of the fragment to the genome map of *C. glutamicum*, and, when performed multiple times with different enzymes, facilitates a rapid determination of the nucleic acid sequence to which the protein binds. Further, the nucleic acid molecules of the invention may be sufficiently homologous to the sequences of related species such that these nucleic acid molecules may serve as markers for the construction of a genomic map in related bacteria, such as *Brevibacterium lactofermentum*.

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The MP nucleic acid molecules of the invention are also useful for evolutionary and protein structural studies. The metabolic processes in which the molecules of the invention participate are utilized by a wide variety of prokaryotic and eukaryotic cells; by comparing the sequences of the nucleic acid molecules of the present invention to those encoding similar enzymes from other organisms, the evolutionary relatedness of the organisms can be assessed. Similarly, such a comparison permits an assessment of which regions of the sequence are conserved and which are not, which may aid in determining those regions of the protein which are essential for the functioning of the enzyme. This type of determination is of value for protein engineering studies and may give an indication of what the protein can tolerate in terms of mutagenesis without losing function.

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Manipulation of the MP nucleic acid molecules of the invention may result in the production of MP proteins having functional differences from the wild-type MP proteins. These proteins may be improved in efficiency or activity, may be present in greater numbers in the cell than is usual, or may be decreased in efficiency or activity.

The invention also provides methods for screening molecules which modulate the activity of an MP protein, either by interacting with the protein itself or a substrate or binding partner of the MP protein, or by modulating the transcription or translation of an MP nucleic acid molecule of the invention. In such methods, a microorganism expressing one or more MP proteins of the invention is contacted with one or more test compounds, and the effect of each test compound on the activity or level of expression of the MP protein is assessed.

When the desired fine chemical to be isolated from large-scale fermentative culture of *C. glutamicum* is an amino acid, a vitamin, a cofactor, a nutraceutical, a nucleotide, a nucleoside, or trehalose, modulation of the activity or efficiency of activity of one or more of the proteins of the invention by recombinant genetic mechanisms may directly impact the production of one of these fine chemicals. For example, in the case of an enzyme in a biosynthetic pathway for a desired amino acid, improvement in efficiency or activity of the enzyme (including the presence of multiple copies of the gene) should lead to an increased production or efficiency of production of that desired amino acid. In the case of an enzyme in a biosynthetic pathway for an amino acid whose synthesis is in competition with the synthesis of a desired amino acid, any decrease in the efficiency or activity of this enzyme (including deletion of the gene) should result in an increase in production or efficiency of production of the desired amino acid, due to decreased competition for intermediate compounds and/or energy. In the case of an enzyme in a degradation pathway for a desired amino acid, any decrease in efficiency or activity of the enzyme should result in a greater yield or efficiency of production of the desired product due to a decrease in its degradation. Lastly, mutagenesis of an enzyme involved in the biosynthesis of a desired amino acid such that this enzyme is no longer capable of feedback inhibition should result in increased yields or efficiency of production of the desired amino acid. The same should apply to the biosynthetic and degradative enzymes of the invention involved in the metabolism of vitamins, cofactors, nutraceuticals, nucleotides, nucleosides and trehalose.

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Similarly, when the desired fine chemical is not one of the
aforementioned compounds, the modulation of activity of one of
the proteins of the invention may still impact the yield and/or
efficiency of production of the compound from large-scale culture
5 of *C. glutamicum*. The metabolic pathways of any organism are
closely interconnected; the intermediate used by one pathway is
often supplied by a different pathway. Enzyme expression and
function may be regulated based on the cellular levels of a
compound from a different metabolic process, and the cellular
10 levels of molecules necessary for basic growth, such as amino
acids and nucleotides, may critically affect the viability of
the microorganism in large-scale culture. Thus, modulation of an
amino acid biosynthesis enzyme, for example, such that it is
no longer responsive to feedback inhibition or such that it
15 is improved in efficiency or turnover may result in increased
cellular levels of one or more amino acids. In turn, this
increased pool of amino acids provides not only an increased
supply of molecules necessary for protein synthesis, but also of
molecules which are utilized as intermediates and precursors in
20 a number of other biosynthetic pathways. If a particular amino
acid had been limiting in the cell, its increased production
might increase the ability of the cell to perform numerous
other metabolic reactions, as well as enabling the cell to more
efficiently produce proteins of all kinds, possibly increasing
25 the overall growth rate or survival ability of the cell in large
scale culture. Increased viability improves the number of cells
capable of producing the desired fine chemical in fermentative
culture, thereby increasing the yield of this compound. Similar
processes are possible by the modulation of activity of a
30 degradative enzyme of the invention such that the enzyme no
longer catalyzes, or catalyzes less efficiently, the degradation
of a cellular compound which is important for the biosynthesis
of a desired compound, or which will enable the cell to grow and
reproduce more efficiently in large-scale culture. It should be
35 emphasized that optimizing the degradative activity or decreasing
the biosynthetic activity of certain molecules of the invention
may also have a beneficial effect on the production of certain
fine chemicals from *C. glutamicum*. For example, by decreasing
the efficiency of activity of a biosynthetic enzyme in a
40 pathway which competes with the biosynthetic pathway of a
desired compound for one or more intermediates, more of those
intermediates should be available for conversion to the desired
product. A similar situation may call for the improvement of
degradative ability or efficiency of one or more proteins of
45 the invention.

This aforementioned list of mutagenesis strategies for MP proteins to result in increased yields of a desired compound is not meant to be limiting; variations on these mutagenesis strategies will be readily apparent to one of ordinary skill in the art. By these mechanisms, the nucleic acid and protein molecules of the invention may be utilized to generate *C. glutamicum* or related strains of bacteria expressing mutated MP nucleic acid and protein molecules such that the yield, production, and/or efficiency of production of a desired compound is improved. This desired compound may be any natural product of *C. glutamicum*, which includes the final products of biosynthesis pathways and intermediates of naturally-occurring metabolic pathways, as well as molecules which do not naturally occur in the metabolism of *C. glutamicum*, but which are produced by a *C. glutamicum* strain of the invention. Preferred compounds to be produced by *Corynebacterium glutamicum* strains are trehalose and/or the amino acids L-lysine and L-methionine.

In one embodiment the *metC* gene encoding cystathionine α -lyase, the third enzyme in the methionine biosynthetic pathway, was isolated from *Corynebacterium glutamicum*. The translational product of the gene showed no significant homology with that of *metC* gene from other organisms. Introduction of the plasmid containing the *metC* gene into *C. glutamicum* resulted in 5-fold increase in the activity of cystathionine α -lyase. The protein product now designated MetC encoding a protein product of 35,574 Dalton consisted of 325 amino acids was identical to the previously reported *aecD* gene except the existence of two different amino acids. Like *aecD* gene, when present in multiple copies, *metC* gene conferred resistance to *S*-(α -aminoethyl)-cysteine which is a toxic lysine analog. However, genetic and biochemical evidences suggest that the natural activity of *metC* gene product is to mediate methionine biosynthesis in *C. glutamicum*. Mutant strains of *metC* were constructed and the strains showed methionine prototrophy. The mutant strains completely lost their ability to show resistance to *S*-(α -aminoethyl)-cysteine. These results show that, in addition to the transsulfuration, another biosynthetic pathway - the direct sulfhydrylation pathway is functional in *C. glutamicum* as a parallel biosynthetic route for methionine.

In yet another embodiment it is also shown that the additional sulfhydrylation pathway is catalyzed by *O*-acetylhomoserine sulfhydrylase. The presence of the pathway is demonstrated by the isolation of the corresponding *metZ* (or *metY*) gene and enzyme. Among the eukaryotes, fungi and yeast species have been reported to have both the transsulfuration and direct sulfhydrylation

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pathway (Marzluf, 1997). So far, no prokaryotic organism which possesses both pathways has been found. Unlike *E. coli* which only possesses single biosynthetic route for lysine, *C. glutamicum* possesses two parallel biosynthetic pathways for the amino acid.

- 5 The biosynthetic pathway for methionine in *C. glutamicum* is analogous to that of lysine in that aspect.

The Gene *metZ* was found because it was located in the upstream region of *metA*. We sequenced regions upstream and downstream of
10 *metA* - the gene encoding the enzyme catalysing the first step of methionine biosynthesis (Park, S.-D., Lee, J.-Y., Kim, Y., Kim, J.-H., and Lee, H.-S. (1998) Isolation and analysis of *metA*, a methionine biosynthetic gene encoding homoserine acetyltransferase in *Corynebacterium glutamicum*. *Mol. Cells* 8,
15 286-294) - to find possible other *met* genes. It appears that *metZ* and *metA* form an operon. Expression of the genes encoding *MetA* and *MetZ* leads to overproduction of the corresponding polypeptides as can shown by gel electrophoresis.

- 20 Surprisingly, *metZ* clones can complement methionine auxotrophic *Escherichia coli metB* mutant strains. This shows that the protein product of *metZ* catalyzes a step that can bypass the step catalyzed by the protein product of *metB*.

- 25 *MetZ* was also disrupted and the mutant strain showed methionine prototrophy. *Corynebacterium glutamicum metB* and *metZ* double mutants were also constructed. The double mutant is auxotrophic for methionine. Thus, *metZ* encodes a protein catalysing the reaction from O-Acetyl-Homoserine to Homocysteine, which is one
30 step in the sulfhydrylation pathway of methionine biosynthesis. *Corynebacterium glutamicum* contains both, the transsulfuration and the sulfhydrylation pathway of methionine biosynthesis.

- Introduction of *metZ* into *C. glutamicum* resulted in the
35 expression of a 47,000 Dalton protein. Combined introduction of *metZ* and *metA* in *C. glutamicum* resulted in the appearance of *metA* and *metZ* proteins as showed by gel electrophoresis. If the *Corynebacterium* strain is a lysine overproducer, introduction of a plasmid containing *metZ* and *metA* resulted in a lower
40 lysine titer but accumulation of homocysteine and methionine is detected.

- In another embodiment *metZ* and *metA* were introduced into *Corynebacterium glutamicum* strains together with the *hom* gene,
45 encoding the homoserine dehydrogenase, catalysing the conversion from aspartate semialdehyde to homoserine. Different *hom* genes from different organisms were chosen for this experiment. The

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Corynebacterium glutamicum *hom* gene can be used as well as *hom* genes from other procaryotes like *Escherichia coli* or *Bacillus subtilis* or even the *hom* gene of eukaryotes like *Saccharomyces cerevisiae*, *Shizosaccharomyces pombe*, *Ashbya gossypii* or algae, higher plants or animals. It may be that the *hom* gene is insensitive against feed back inhibition mediated by any metabolites that occur in the biosynthetic routes of the amino acids of the aspartate family, like aspartate, lysine, threonine or methionine. Such metabolites are for example aspartate, lysine, methionine, threonine, aspartyl-phosphate, aspartate semialdehyde, homoserine, cystathionine, homocysteine or any other metabolite that occurs in this biosynthetic routes. In addition to the metabolites the homoserine dehydrogenase may be insensitive against inhibition by analogues of all those metabolites or even against other compounds involved in this metabolism as there are other amino acids like cysteine or cofactors like vitamin B12 and all of its derivatives and S-adenosylmethionine and its metabolites and derivatives and analogues. The insensitivity of the homoserine dehydrogenase against all these, a part of these or only one of these compounds may either be its natural attitude or it may be the result from one or more mutations that resulted from classical mutation and selection using chemicals or irradiation or other mutagens. The mutations could also be introduced into the *hom* gene using gene technology, for example the introduction of site specific point mutations or by any method afore mentioned for the MP or MP encoding DNA-sequences.

When a *hom* gene was combined with the *metZ* and *metA* genes and introduced into a *Corynebacterium glutamicum* strain that is a lysine overproducer, lysine accumulation was reduced and homocysteine and methionine accumulation was enhanced. A further enhancement of homocysteine and methionine concentrations can be achieved, if a lysine overproducing *Corynebacterium glutamicum* strain is used and a disruption of the *ddh* gene or the *lysA* gene was introduced prior to the transformation with DNA containing a *hom* gene and *metZ* and *metA* in combination. The overproduction of homocysteine and methionine was possible using different sulfur sources. Sulfates, thiosulfates, sulfites and also more reduced sulfur sources like H_2S and sulfides and derivatives could be used. Also organic sulfur sources like methyl mercaptan, thioglycolates, thiocyanates, thiourea, sulfur containing amino acids like cysteine and other sulfur containing compounds can be used to achieve homocysteine and methionine overproduction.

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In another embodiment the *metC* gene was introduced into a *Corynebacterium glutamicum* strain using methods which are aforementioned. The *metC* gene can be transformed into the strain in combination with other genes like *metB*, *metA* and *metA*. Even
5 the *hom* gene can be added. If the *hom* gene, the *met C*, *metA* and *metB* genes were combined on a vector and introduced into a *Corynebacterium glutamicum* strain homocysteine and methionine overproduction was achieved. The overproduction of homocysteine and methionine was possible using different sulfur sources.
10 Sulfates, thiosulfates, sulfites and also more reduced sulfur sources like H_2S and sulfides and derivatives could be used. Also organic sulfur sources like methyl mercaptan, thioglycolates, thiocyanates, thiourea, sulfur containing amino acids like cysteine and other sulfur containing compounds can be used
15 to achieve homocysteine and methionine overproduction.

This invention is further illustrated by the following examples which should not be construed as limiting. The contents of all references, patent applications, patents, published patent
20 applications, Tables, and the sequence listing cited throughout this application are hereby incorporated by reference.

Example 1: Preparation of total genomic DNA of *Corynebacterium glutamicum* ATCC 13032

25 A culture of *Corynebacterium glutamicum* (ATCC 13032) was grown overnight at 30°C with vigorous shaking in BHI medium (Difco). The cells were harvested by centrifugation, the supernatant was discarded and the cells were resuspended in 5 ml buffer-I (5% of
30 the original volume of the culture - all indicated volumes have been calculated for 100 ml of culture volume). Composition of buffer-I: 140.34 g/l sucrose, 2.46 g/l $MgSO_4 \times 7H_2O$, 10 ml/l KH_2PO_4 solution (100 g/l, adjusted to pH 6.7 with KOH), 50 ml/l M12 concentrate (10 g/l $(NH_4)_2SO_4$, 1 g/l NaCl, 2 g/l $MgSO_4 \times 7H_2O$,
35 0.2 g/l $CaCl_2$, 0.5 g/l yeast extract (Difco), 10 ml/l trace-elements-mix (200 mg/l $FeSO_4 \times H_2O$, 10 mg/l $ZnSO_4 \times 7 H_2O$, 3 mg/l $MnCl_2 \times 4 H_2O$, 30 mg/l H_3BO_3 , 20 mg/l $CoCl_2 \times 6 H_2O$, 1 mg/l $NiCl_2 \times 6 H_2O$, 3 mg/l $Na_2MoO_4 \times 2 H_2O$, 500 mg/l complexing agent (EDTA or citric acid), 100 ml/l vitamins-mix (0.2 mg/l biotin, 0.2 mg/l
40 folic acid, 20 mg/l p-amino benzoic acid, 20 mg/l riboflavin, 40 mg/l ca-pantothenate, 140 mg/l nicotinic acid, 40 mg/l pyridoxole hydrochloride, 200 mg/l myo-inositol). Lysozyme was added to the suspension to a final concentration of 2.5 mg/ml. After an approximately 4 h incubation at 37°C, the cell wall
45 was degraded and the resulting protoplasts are harvested by centrifugation. The pellet was washed once with 5 ml buffer-I and once with 5 ml TE-buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8). The

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pellet was resuspended in 4 ml TE-buffer and 0.5 ml SDS solution (10%) and 0.5 ml NaCl solution (5 M) are added. After adding of proteinase K to a final concentration of 200 µg/ml, the suspension is incubated for ca.18 h at 37°C. The DNA was purified
5 by extraction with phenol, phenol-chloroform-isoamylalcohol and chloroform-isoamylalcohol using standard procedures. Then, the DNA was precipitated by adding 1/50 volume of 3 M sodium acetate and 2 volumes of ethanol, followed by a 30 min incubation at -20°C and a 30 min centrifugation at 12,000 rpm in a high speed
10 centrifuge using a SS34 rotor (Sorvall). The DNA was dissolved in 1 ml TE-buffer containing 20 µg/ml RNaseA and dialysed at 4°C against 1000 ml TE-buffer for at least 3 hours. During this time, the buffer was exchanged 3 times. To aliquots of 0.4 ml of the dialysed DNA solution, 0.4 ml of 2 M LiCl and 0.8 ml of ethanol
15 are added. After a 30 min incubation at -20°C, the DNA was collected by centrifugation (13,000 rpm, Biofuge Fresco, Heraeus, Hanau, Germany). The DNA pellet was dissolved in TE-buffer. DNA prepared by this procedure could be used for all purposes, including southern blotting or construction of genomic libraries.

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Example 2: Construction of genomic libraries in *Escherichia coli* of *Corynebacterium glutamicum* ATCC13032.

Using DNA prepared as described in Example 1, cosmid and plasmid
25 libraries were constructed according to known and well established methods (see e.g., Sambrook, J. et al. (1989) "Molecular Cloning : A Laboratory Manual", Cold Spring Harbor Laboratory Press, or Ausubel, F.M. et al. (1994) "Current Protocols in Molecular Biology", John Wiley & Sons.)

30

Any plasmid or cosmid could be used. Of particular use were the plasmids pBR322 (Sutcliffe, J.G. (1979) *Proc. Natl. Acad. Sci. USA*, 75:3737-3741); pACYC177 (Change & Cohen (1978) *J. Bacteriol* 134:1141-1156), plasmids of the pBS series (pBSSK+, pBSSK- and
35 others; Stratagene, LaJolla, USA), or cosmids as SuperCos1 (Stratagene, LaJolla, USA) or Lorist6 (Gibson, T.J., Rosenthal A. and Waterson, R.H. (1987) *Gene* 53:283-286. Gene libraries specifically for use in *C. glutamicum* may be constructed using plasmid pSL109 (Lee, H.-S. and A. J. Sinskey (1994) *J. Microbiol.*
40 *Biotechnol.* 4: 256-263).

Example 3: DNA Sequencing and Computational Functional Analysis

Genomic libraries as described in Example 2 were used for DNA
45 sequencing according to standard methods, in particular by the chain termination method using ABI377 sequencing machines (see e.g., Fleischman, R.D. et al. (1995) "Whole-genome Random

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Sequencing and Assembly of Haemophilus Influenzae Rd., *Science*, 269:496-512). Sequencing primers with the following nucleotide sequences were used: 5'-GGAAACAGTATGACCATG-3' or 5'-GTAAAACGACGGCCAGT-3'.

5

Example 4: In vivo Mutagenesis

In vivo mutagenesis of *Corynebacterium glutamicum* can be performed by passage of plasmid (or other vector) DNA through *E. coli* or other microorganisms (e.g. *Bacillus* spp. or yeasts such as *Saccharomyces cerevisiae*) which are impaired in their capabilities to maintain the integrity of their genetic information. Typical mutator strains have mutations in the genes for the DNA repair system (e.g., mutHLS, mutD, mutT, etc.; for reference, see Rupp, W.D. (1996) DNA repair mechanisms, in: *Escherichia coli* and *Salmonella*, p. 2277-2294, ASM: Washington.) Such strains are well known to those of ordinary skill in the art. The use of such strains is illustrated, for example, in Greener, A. and Callahan, M. (1994) *Strategies* 7: 32-34.

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Example 5: DNA Transfer Between *Escherichia coli* and *Corynebacterium glutamicum*

Several *Corynebacterium* and *Brevibacterium* species contain endogenous plasmids (as e.g., pHM1519 or pBL1) which replicate autonomously (for review see, e.g., Martin, J.F. et al. (1987) *Biotechnology*, 5:137-146). Shuttle vectors for *Escherichia coli* and *Corynebacterium glutamicum* can be readily constructed by using standard vectors for *E. coli* (Sambrook, J. et al. (1989), "Molecular Cloning: A Laboratory Manual", Cold Spring Harbor Laboratory Press or Ausubel, F.M. et al. (1994) "Current Protocols in Molecular Biology", John Wiley & Sons) to which a origin or replication for and a suitable marker from *Corynebacterium glutamicum* is added. Such origins of replication are preferably taken from endogenous plasmids isolated from *Corynebacterium* and *Brevibacterium* species. Of particular use as transformation markers for these species are genes for kanamycin resistance (such as those derived from the Tn5 or Tn903 transposons) or chloramphenicol (Winnacker, E.L. (1987) "From Genes to Clones - Introduction to Gene Technology, VCH, Weinheim). There are numerous examples in the literature of the construction of a wide variety of shuttle vectors which replicate in both *E. coli* and *C. glutamicum*, and which can be used for several purposes, including gene over-expression (for reference, see e.g., Yoshihama, M. et al. (1985) *J. Bacteriol.* 162:591-597,

Martin J.F. et al. (1987) *Biotechnology*, 5:137-146 and Eikmanns, B.J. et al. (1991) *Gene*, 102:93-98).

- Using standard methods, it is possible to clone a gene of
5 interest into one of the shuttle vectors described above and to
introduce such a hybrid vectors into strains of *Corynebacterium
glutamicum*. Transformation of *C. glutamicum* can be achieved by
protoplast transformation (Kastsumata, R. et al. (1984) *J.
Bacteriol.* 159:306-311), electroporation (Liebl, E. et al. (1989)
10 *FEMS Microbiol. Letters*, 53:399-303) and in cases where special
vectors are used, also by conjugation (as described e.g. in
Schäfer, A et al. (1990) *J. Bacteriol.* 172:1663-1666). It is also
possible to transfer the shuttle vectors for *C. glutamicum* to *E.
coli* by preparing plasmid DNA from *C. glutamicum* (using standard
15 methods well-known in the art) and transforming it into *E. coli*.
This transformation step can be performed using standard methods,
but it is advantageous to use an *Mcr*-deficient *E. coli* strain,
such as NM522 (Gough & Murray (1983) *J. Mol. Biol.* 166:1-19).
- 20 Genes may be overexpressed in *C. glutamicum* strains using
plasmids which comprise pCG1 (U.S. Patent No. 4,617,267) or
fragments thereof, and optionally the gene for kanamycin
resistance from TN903 (Grindley, N.D. and Joyce, C.M. (1980)
Proc. Natl. Acad. Sci. USA 77(12): 7176-7180). In addition,
25 genes may be overexpressed in *C. glutamicum* strains using plasmid
pSL109 (Lee, H.-S. and A. J. Sinskey (1994) *J. Microbiol.
Biotechnol.* 4: 256-263).

- Aside from the use of replicative plasmids, gene overexpression
30 can also be achieved by integration into the genome. Genomic
integration in *C. glutamicum* or other *Corynebacterium* or
Brevibacterium species may be accomplished by well-known methods,
such as homologous recombination with genomic region(s),
restriction endonuclease mediated integration (REMI) (see, e.g.,
35 DE Patent 19823834), or through the use of transposons. It is
also possible to modulate the activity of a gene of interest by
modifying the regulatory regions (e.g., a promoter, a repressor,
and/or an enhancer) by sequence modification, insertion, or
deletion using site-directed methods (such as homologous
40 recombination) or methods based on random events (such as
transposon mutagenesis or REMI). Nucleic acid sequences which
function as transcriptional terminators may also be inserted 3'
to the coding region of one or more genes of the invention;
such terminators are well-known in the art and are described,
45 for example, in Winnacker, E.L. (1987) *From Genes to Clones -
Introduction to Gene Technology*. VCH: Weinheim.

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Example 6: Assessment of the Expression of the Mutant Protein

Observations of the activity of a mutated protein in a transformed host cell rely on the fact that the mutant protein is expressed in a similar fashion and in a similar quantity to that of the wild-type protein. A useful method to ascertain the level of transcription of the mutant gene (an indicator of the amount of mRNA available for translation to the gene product) is to perform a Northern blot (for reference see, for example, Ausubel et al. (1988) Current Protocols in Molecular Biology, Wiley: New York), in which a primer designed to bind to the gene of interest is labeled with a detectable tag (usually radioactive or chemiluminescent), such that when the total RNA of a culture of the organism is extracted, run on gel, transferred to a stable matrix and incubated with this probe, the binding and quantity of binding of the probe indicates the presence and also the quantity of mRNA for this gene. This information is evidence of the degree of transcription of the mutant gene. Total cellular RNA can be prepared from *Corynebacterium glutamicum* by several methods, all well-known in the art, such as that described in Bormann, E.R. et al. (1992) *Mol. Microbiol.* 6: 317-326.

To assess the presence or relative quantity of protein translated from this mRNA, standard techniques, such as a SDS-Polyacrylamide Gelelectrophoresis and Western blot, may be employed (see, for example, Ausubel et al. (1988) Current Protocols in Molecular Biology, Wiley: New York). In this process, total cellular proteins are extracted, separated by gel electrophoresis, transferred to a matrix such as nitrocellulose, and incubated with a probe, such as an antibody, which specifically binds to the desired protein. This probe is generally tagged with a chemiluminescent or colorimetric label which may be readily detected. The presence and quantity of label observed indicates the presence and quantity of the desired mutant protein present in the cell.

Example 7: Growth of *Escherichia coli* and Genetically Modified *Corynebacterium glutamicum* - Media and Culture Conditions

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E. coli strains are routinely grown in MB and LB broth, respectively (Follettie, M.T., Peoples, O., Agoropoulou, C., and Sinskey, A.J. (1993) Gene structure and expression of the *Corynebacterium flavum* N13 ask-asd operon. *J. Bacteriol.* 175, 4096-4103). Minimal media for *E. coli* is M9 and modified MCGC (Yoshihama, M., Higashiro, K., Rao, E.A., Akedo, M., Shanabruch, W.G., Follettie, M.T., Walker, G.C., and Sinskey, A.J.

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(1985) Cloning vector system for *Corynebacterium glutamicum*. J. Bacteriol. 162, 591-507), respectively. Glucose was added a final concentration of 1%. Antibiotics were added in the following amounts (micrograms per milliliter): ampicillin, 50; 5 kanamycin, 25; nalidixic acid, 25. Amino acids, vitamins, and other supplements were added in the following amounts: methionine, 9.3 mM; arginine, 9.3 mM; histidine, 9.3 mM; thiamine, 0.05 mM. E. coli cells were routinely grown at 37°C, respectively.

10

- Genetically modified *Corynebacteria* are cultured in synthetic or natural growth media. A number of different growth media for *Corynebacteria* are both well-known and readily available (Lieb et al. (1989) Appl. Microbiol. Biotechnol., 32:205-210; von der 15 Osten et al. (1998) Biotechnology Letters, 11:11-16; Patent DE 4,120,867; Liebl (1992) "The Genus *Corynebacterium*, in: The Procaryotes, Volume II, Balows, A. et al., eds. Springer-Verlag). These media consist of one or more carbon sources, nitrogen sources, inorganic salts, vitamins and trace elements.
- 20 Preferred carbon sources are sugars, such as mono-, di-, or polysaccharides. For example, glucose, fructose, mannose, galactose, ribose, sorbose, ribulose, lactose, maltose, sucrose, raffinose, starch or cellulose serve as very good carbon sources. It is also possible to supply sugar to the media via complex 25 compounds such as molasses or other by-products from sugar refinement. It can also be advantageous to supply mixtures of different carbon sources. Other possible carbon sources are alcohols and organic acids, such as methanol, ethanol, acetic acid or lactic acid. Nitrogen sources are usually organic or 30 inorganic nitrogen compounds, or materials which contain these compounds. Exemplary nitrogen sources include ammonia gas or ammonia salts, such as NH_4Cl or $(\text{NH}_4)_2\text{SO}_4$, NH_4OH , nitrates, urea, amino acids or complex nitrogen sources like corn steep liquor, soy bean flour, soy bean protein, yeast extract, meat extract 35 and others.

The overproduction of sulfur containing amino acids like homocysteine and methionine was possible using different sulfur sources. Sulfates, thiosulfates, sulfites and also more reduced 40 sulfur sources like H_2S and sulfides and derivatives can be used. Also organic sulfur sources like methyl mercaptan, thioglycolates, thiocyanates, thiourea, sulfur containing amino acids like cysteine and other sulfur containing compounds can be used to achieve homocysteine and methionine overproduction.

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Inorganic salt compounds which may be included in the media include the chloride-, phosphorous- or sulfate- salts of calcium, magnesium, sodium, cobalt, molybdenum, potassium, manganese, zinc, copper and iron. Chelating compounds can be added to the
5 medium to keep the metal ions in solution. Particularly useful chelating compounds include dihydroxyphenols, like catechol or protocatechuate, or organic acids, such as citric acid. It is typical for the media to also contain other growth factors, such as vitamins or growth promoters, examples of which include
10 biotin, riboflavin, thiamin, folic acid, nicotinic acid, pantothenate and pyridoxin. Growth factors and salts frequently originate from complex media components such as yeast extract, molasses, corn steep liquor and others. The exact composition of the media compounds depends strongly on the immediate experiment
15 and is individually decided for each specific case. Information about media optimization is available in the textbook "Applied Microbiol. Physiology, A Practical Approach (eds. P.M. Rhodes, P.F. Stanbury, IRL Press (1997) pp. 53-73, ISBN 0 19 963577 3). It is also possible to select growth media from commercial
20 suppliers, like standard 1 (Merck) or BHI (grain heart infusion, DIFCO) or others.

All medium components are sterilized, either by heat (20 minutes at 1.5 bar and 121°C) or by sterile filtration. The components
25 can either be sterilized together or, if necessary, separately. All media components can be present at the beginning of growth, or they can optionally be added continuously or batchwise.

Culture conditions are defined separately for each experiment.
30 The temperature should be in a range between 15°C and 45°C. The temperature can be kept constant or can be altered during the experiment. The pH of the medium should be in the range of 5 to 8.5, preferably around 7.0, and can be maintained by the addition of buffers to the media. An exemplary buffer for this purpose
35 is a potassium phosphate buffer. Synthetic buffers such as MOPS, HEPES, ACES and others can alternatively or simultaneously be used. It is also possible to maintain a constant culture pH through the addition of NaOH or NH₄OH during growth. If complex medium components such as yeast extract are utilized, the
40 necessity for additional buffers may be reduced, due to the fact that many complex compounds have high buffer capacities. If a fermentor is utilized for culturing the micro-organisms, the pH can also be controlled using gaseous ammonia.

45 The incubation time is usually in a range from several hours to several days. This time is selected in order to permit the maximal amount of product to accumulate in the broth. The

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disclosed growth experiments can be carried out in a variety of vessels, such as microtiter plates, glass tubes, glass flasks or glass or metal fermentors of different sizes. For screening a large number of clones, the microorganisms should be cultured
5 in microtiter plates, glass tubes or shake flasks, either with or without baffles. Preferably 100 ml shake flasks are used, filled with 10% (by volume) of the required growth medium. The flasks should be shaken on a rotary shaker (amplitude 25 mm) using a speed-range of 100-300 rpm. Evaporation losses
10 can be diminished by the maintenance of a humid atmosphere; alternatively, a mathematical correction for evaporation losses should be performed.

If genetically modified clones are tested, an unmodified control
15 clone or a control clone containing the basic plasmid without any insert should also be tested. The medium is inoculated to an OD₆₀₀ of 0.5 - 1.5 using cells grown on agar plates, such as CM plates (10 g/l glucose, 2,5 g/l NaCl, 2 g/l urea, 10 g/l polypeptone, 5 g/l yeast extract, 5 g/l meat extract, 22 g/l NaCl, 2 g/l urea,
20 10 g/l polypeptone, 5 g/l yeast extract, 5 g/l meat extract, 22 g/l agar, pH 6.8 with 2M NaOH) that had been incubated at 30°C. Inoculation of the media is accomplished by either introduction of a saline suspension of *C. glutamicum* cells from CM plates or addition of a liquid preculture of this bacterium.

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Example 8: *In vitro* Analysis of the Function of Mutant Proteins

The determination of activities and kinetic parameters of enzymes is well established in the art. Experiments to determine the
30 activity of any given altered enzyme must be tailored to the specific activity of the wild-type enzyme, which is well within the ability of one of ordinary skill in the art. Overviews about enzymes in general, as well as specific details concerning structure, kinetics, principles, methods, applications and
35 examples for the determination of many enzyme activities may be found, for example, in the following references: Dixon, M., and Webb, E.C., (1979) *Enzymes*. Longmans: London; Fersht, (1985) *Enzyme Structure and Mechanism*. Freeman: New York; Walsh, (1979) *Enzymatic Reaction Mechanisms*. Freeman: San Francisco; Price,
40 N.C., Stevens, L. (1982) *Fundamentals of Enzymology*. Oxford Univ. Press: Oxford; Boyer, P.D., ed. (1983) *The Enzymes*, 3rd ed. Academic Press: New York; Bisswanger, H., (1994) *Enzymkinetik*, 2nd ed. VCH: Weinheim (ISBN 3527300325); Bergmeyer, H.U., Bergmeyer, J., Graßl, M., eds. (1983-1986) *Methods of Enzymatic*
45 *Analysis*, 3rd ed., vol. I-XII, Verlag Chemie: Weinheim; and

Ullmann's Encyclopedia of Industrial Chemistry (1987) vol. A9, "Enzymes". VCH: Weinheim, p. 352-363.

- The activity of proteins which bind to DNA can be measured by several well-established methods, such as DNA band-shift assays (also called gel retardation assays). The effect of such proteins on the expression of other molecules can be measured using reporter gene assays (such as that described in Kolmar, H. et al. (1995) *EMBO J.* 14: 3895-3904 and references cited therein).
- 10 Reporter gene test systems are well known and established for applications in both pro- and eukaryotic cells, using enzymes such as beta-galactosidase, green fluorescent protein, and several others.
- 15 The determination of activity of membrane-transport proteins can be performed according to techniques such as those described in Gennis, R.B. (1989) "Pores, Channels and Transporters", in Biomembranes, Molecular Structure and Function, Springer: Heidelberg, p. 85-137; 199-234; and 270-322.
- 20 Example 9: Analysis of Impact of Mutant Protein on the Production of the Desired Product

- The effect of the genetic modification in *C. glutamicum* on production of a desired compound (such as an amino acid) can be assessed by growing the modified microorganism under suitable conditions (such as those described above) and analyzing the medium and/or the cellular component for increased production of the desired product (i.e., an amino acid). Such analysis techniques are well known to one of ordinary skill in the art, and include spectroscopy, thin layer chromatography, staining methods of various kinds, enzymatic and microbiological methods, and analytical chromatography such as high performance liquid chromatography (see, for example, Ullman, Encyclopedia of Industrial Chemistry; vol. A2, p. 89-90 and p. 443-613, VCH: Weinheim (1985); Fallon, A. et al., (1987) "Applications of HPLC in Biochemistry" in: Laboratory Techniques in Biochemistry and Molecular Biology, vol. 17; Rehm et al. (1993) Biotechnology, vol. 3, Chapter III: "Product recovery and purification", page 469-714, VCH: Weinheim; Belter, P.A. et al. (1988) Bioseparations: downstream processing for biotechnology, John Wiley and Sons; Kennedy, J.F. and Cabral, J.M.S. (1992) Recovery processes for biological materials, John Wiley and Sons; Shaeiwitz, J.A. and Henry, J.D. (1988) Biochemical separations, in: Ullmann's Encyclopedia of Industrial Chemistry, vol. B3, Chapter 11, page 1-27, VCH: Weinheim; and Dechow, F.J. (1989)

Separation and purification techniques in biotechnology, Noyes Publications.)

In addition to the measurement of the final product of
5 fermentation, it is also possible to analyze other components of
the metabolic pathways utilized for the production of the desired
compound, such as intermediates and side-products, to determine
the overall efficiency of production of the compound. Analysis
methods include measurements of nutrient levels in the medium
10 (e.g., sugars, hydrocarbons, nitrogen sources, phosphate, and
other ions), measurements of biomass composition and growth,
analysis of the production of common metabolites of biosynthetic
pathways, and measurement of gasses produced during fermentation.
Standard methods for these measurements are outlined in Applied
15 Microbial Physiology, A Practical Approach, P.M. Rhodes and P.F.
Stanbury, eds., IRL Press, p. 103-129; 131-163; and 165-192
(ISBN: 0199635773) and references cited therein.

20 Example 10: Purification of the Desired Product from *C. glutamicum*
Culture

Recovery of the desired product from the *C. glutamicum* cells or
supernatant of the above-described culture can be performed by
various methods well known in the art. If the desired product is
25 not secreted from the cells, the cells can be harvested from the
culture by low-speed centrifugation, the cells can be lysed by
standard techniques, such as mechanical force or sonication. The
cellular debris is removed by centrifugation, and the supernatant
fraction containing the soluble proteins is retained for further
30 purification of the desired compound. If the product is secreted
from the *C. glutamicum* cells, then the cells are removed from the
culture by low-speed centrifugation, and the supernate fraction
is retained for further purification.

35 The supernatant fraction from either purification method is
subjected to chromatography with a suitable resin, in which the
desired molecule is either retained on a chromatography resin
while many of the impurities in the sample are not, or where the
impurities are retained by the resin while the sample is not.
40 Such chromatography steps may be repeated as necessary, using the
same or different chromatography resins. One of ordinary skill
in the art would be well-versed in the selection of appropriate
chromatography resins and in their most efficacious application
for a particular molecule to be purified. The purified product
45 may be concentrated by filtration or ultrafiltration, and stored

at a temperature at which the stability of the product is maximized.

- There are a wide array of purification methods known to the art and the preceding method of purification is not meant to be limiting. Such purification techniques are described, for example, in Bailey, J.E. & Ollis, D.F. *Biochemical Engineering Fundamentals*, McGraw-Hill: New York (1986).
- 10 The identity and purity of the isolated compounds may be assessed by techniques standard in the art. These include high-performance liquid chromatography (HPLC), spectroscopic methods, staining methods, thin layer chromatography, NIRS, enzymatic assay, or microbiologically. Such analysis methods are reviewed in: Patek
15 et al. (1994) *Appl. Environ. Microbiol.* 60: 133-140; Malakhova et al. (1996) *Biotekhnologiya* 11: 27-32; and Schmidt et al. (1998) *Bioprocess Engineer.* 19: 67-70. *Ulmann's Encyclopedia of Industrial Chemistry*, (1996) vol. A27, VCH: Weinheim, p. 89-90, p. 521-540, p. 540-547, p. 559-566, 575-581 and p. 581-587;
20 Michal, G. (1999) *Biochemical Pathways: An Atlas of Biochemistry and Molecular Biology*, John Wiley and Sons; Fallon, A. et al. (1987) *Applications of HPLC in Biochemistry in: Laboratory Techniques in Biochemistry and Molecular Biology*, vol. 17.
- 25 Example 11: Analysis of the Gene Sequences of the Invention
- The comparison of sequences and determination of percent homology between two sequences are art-known techniques, and can be accomplished using a mathematical algorithm, such as the
30 algorithm of Karlin and Altschul (1990) *Proc. Natl. Acad. Sci. USA* 87:2264-68, modified as in Karlin and Altschul (1993) *Proc. Natl. Acad. Sci. USA* 90:5873-77. Such an algorithm is incorporated into the NBLAST and XBLAST programs (version 2.0) of Altschul, et al. (1990) *J. Mol. Biol.* 215:403-10. BLAST
35 nucleotide searches can be performed with the NBLAST program, score = 100, wordlength = 12 to obtain nucleotide sequences homologous to MP nucleic acid molecules of the invention. BLAST protein searches can be performed with the XBLAST program, score = 50, wordlength = 3 to obtain amino acid sequences homologous
40 to MP protein molecules of the invention. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul et al., (1997) *Nucleic Acids Res.* 25(17):3389-3402. When utilizing BLAST and Gapped BLAST programs, one of ordinary skill in the art will know how to optimize the
45 parameters of the program (e.g., XBLAST and NBLAST) for the specific sequence being analyzed.

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Another example of a mathematical algorithm utilized for the comparison of sequences is the algorithm of Meyers and Miller ((1988) *Comput. Appl. Biosci.* 4: 11-17). Such an algorithm is incorporated into the ALIGN program (version 2.0) which is part
5 of the GCG sequence alignment software package. When utilizing the ALIGN program for comparing amino acid sequences, a PAM120 weight residue table, a gap length penalty of 12, and a gap penalty of 4 can be used. Additional algorithms for sequence analysis are known in the art, and include ADVANCE and ADAM.
10 described in Torelli and Robotti (1994) *Comput. Appl. Biosci.* 10:3-5; and FASTA, described in Pearson and Lipman (1988) *P.N.A.S.* 85:2444-8.

The percent homology between two amino acid sequences can also be
15 accomplished using the GAP program in the GCG software package (available at <http://www.gcg.com>), using either a Blosum 62 matrix or a PAM250 matrix, and a gap weight of 12, 10, 8, 6, or 4 and a length weight of 2, 3, or 4. The percent homology between two nucleic acid sequences can be accomplished using the GAP
20 program in the GCG software package, using standard parameters, such as a gap weight of 50 and a length weight of 3.

A comparative analysis of the gene sequences of the invention with those present in Genbank has been performed using techniques
25 known in the art (see, e.g., Bexevanis and Ouellette, eds. (1998) *Bioinformatics: A Practical Guide to the Analysis of Genes and Proteins*. John Wiley and Sons: New York).

The gene sequences of the invention were compared on basis of
30 their amino acid sequences to known genes by using the program CLUSTAL (Higgins et al. (1996) *Using CLUSTAL for multiple sequence alignments, Methods in Enzymology* 266, 383-402) using the standard parameters (PAIRWISE ALIGNMENT PARAMETERS: Gap penalty= 3, K-tuple (word) size= 1, No. of top diagonals= 5,
35 Window size= 5; MULTIPLE ALIGNMENT PARAMETERS: Gap Opening Penalty= 10.00, Gap Extension Penalty= 0.05, Protein weight matrix= PAM250). Homology between two sequences is the function of the number of identical positions in all sequences (i.e.
% homology = number of identical positions/total number of
40 positions x 100). The results of this analysis are set forth in Table 3.

Example 12: Construction and Operation of DNA Microarrays

45 The sequences of the invention may additionally be used in the construction and application of DNA microarrays (the design, methodology, and uses of DNA arrays are well known in the art,

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and are described, for example, in Schena, M. et al. (1995) *Science* 270: 467-470; Wodicka, L. et al. (1997) *Nature Biotechnology* 15: 1359-1367; DeSaizieu, A. et al. (1998) *Nature Biotechnology* 16: 45-48; and DeRisi, J.L. et al. (1997) *Science* 5 278: 680-686).

DNA microarrays are solid or flexible supports consisting of nitrocellulose, nylon, glass, silicone, or other materials. Nucleic acid molecules may be attached to the surface in an
10 ordered manner. After appropriate labeling, other nucleic acids or nucleic acid mixtures can be hybridized to the immobilized nucleic acid molecules, and the label may be used to monitor and measure the individual signal intensities of the hybridized molecules at defined regions. This methodology allows the
15 simultaneous quantification of the relative or absolute amount of all or selected nucleic acids in the applied nucleic acid sample or mixture. DNA microarrays, therefore, permit an analysis of the expression of multiple (as many as 6800 or more) nucleic acids in parallel (see, e.g., Schena, M. (1996) *BioEssays* 18(5): 427-431).

20 The sequences of the invention may be used to design oligonucleotide primers which are able to amplify defined regions of one or more *C. glutamicum* genes by a nucleic acid amplification reaction such as the polymerase chain reaction.
25 The choice and design of the 5' or 3' oligonucleotide primers or of appropriate linkers allows the covalent attachment of the resulting PCR products to the surface of a support medium described above (and also described, for example, Schena, M. et al. (1995) *Science* 270: 467-470).

30 Nucleic acid microarrays may also be constructed by *in situ* oligonucleotide synthesis as described by Wodicka, L. et al. (1997) *Nature Biotechnology* 15: 1359-1367. By photolithographic methods, precisely defined regions of the matrix are exposed
35 to light. Protective groups which are photolabile are thereby activated and undergo nucleotide addition, whereas regions that are masked from light do not undergo any modification. Subsequent cycles of protection and light activation permit the synthesis of different oligonucleotides at defined positions. Small, defined
40 regions of the genes of the invention may be synthesized on microarrays by solid phase oligonucleotide synthesis.

The nucleic acid molecules of the invention present in a sample or mixture of nucleotides may be hybridized to the microarrays.
45 These nucleic acid molecules can be labeled according to standard methods. In brief, nucleic acid molecules (e.g., mRNA molecules or DNA molecules) are labeled by the incorporation of

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isotopically or fluorescently labeled nucleotides, e.g., during reverse transcription or DNA synthesis. Hybridization of labeled nucleic acids to microarrays is described (e.g., in Schena, M. et al. (1995) *supra*; Wodicka, L. et al. (1997), *supra*; and DeSaizieu
5 A. et al. (1998), *supra*). The detection and quantification of the hybridized molecule are tailored to the specific incorporated label. Radioactive labels can be detected, for example, as described in Schena, M. et al. (1995) *supra*) and fluorescent labels may be detected, for example, by the method of Shalon
10 et al. (1996) *Genome Research* 6: 639-645).

The application of the sequences of the invention to DNA microarray technology, as described above, permits comparative analyses of different strains of *C. glutamicum* or other
15 *Corynebacteria*. For example, studies of inter-strain variations based on individual transcript profiles and the identification of genes that are important for specific and/or desired strain properties such as pathogenicity, productivity and stress tolerance are facilitated by nucleic acid array methodologies.
20 Also, comparisons of the profile of expression of genes of the invention during the course of a fermentation reaction are possible using nucleic acid array technology.

Example 13: Analysis of the Dynamics of Cellular Protein
25 Populations (Proteomics)

The genes, compositions, and methods of the invention may be applied to study the interactions and dynamics of populations of proteins, termed 'proteomics'. Protein populations of interest
30 include, but are not limited to, the total protein population of *C. glutamicum* (e.g., in comparison with the protein populations of other organisms), those proteins which are active under specific environmental or metabolic conditions (e.g., during fermentation, at high or low temperature, or at high or low pH),
35 or those proteins which are active during specific phases of growth and development.

Protein populations can be analyzed by various well-known techniques, such as gel electrophoresis. Cellular proteins may
40 be obtained, for example, by lysis or extraction, and may be separated from one another using a variety of electrophoretic techniques. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) separates proteins largely on the basis of their molecular weight. Isoelectric focusing
45 polyacrylamide gel electrophoresis (IEF-PAGE) separates proteins by their isoelectric point (which reflects not only the amino acid sequence but also posttranslational modifications of the

protein). Another, more preferred method of protein analysis is the consecutive combination of both IEF-PAGE and SDS-PAGE, known as 2-D-gel electrophoresis (described, for example, in Hermann et al. (1998) *Electrophoresis* 19: 3217-3221; Fountoulakis et al. (1998) *Electrophoresis* 19: 1193-1202; Langen et al. (1997) *Electrophoresis* 18: 1184-1192; Antelmann et al. (1997) *Electrophoresis* 18: 1451-1463). Other separation techniques may also be utilized for protein separation, such as capillary gel electrophoresis; such techniques are well known in the art.

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Proteins separated by these methodologies can be visualized by standard techniques, such as by staining or labeling. Suitable stains are known in the art, and include Coomassie Brilliant Blue, silver stain, or fluorescent dyes such as Sypro Ruby

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(Molecular Probes). The inclusion of radioactively labeled amino acids or other protein precursors (e.g., ^{35}S -methionine, ^{35}S -cysteine, ^{14}C -labelled amino acids, ^{15}N -amino acids, $^{15}\text{NO}_3$ or $^{15}\text{NH}_4^+$ or ^{13}C -labelled amino acids) in the medium of *C. glutamicum* permits the labeling of proteins from these cells prior to

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their separation. Similarly, fluorescent labels may be employed. These labeled proteins can be extracted, isolated and separated according to the previously described techniques.

Proteins visualized by these techniques can be further analyzed by measuring the amount of dye or label used. The amount of a given protein can be determined quantitatively using, for example, optical methods and can be compared to the amount of other proteins in the same gel or in other gels. Comparisons of proteins on gels can be made, for example, by optical comparison, by spectroscopy, by image scanning and analysis of gels, or through the use of photographic films and screens. Such techniques are well-known in the art.

To determine the identity of any given protein, direct sequencing or other standard techniques may be employed. For example, N- and/or C-terminal amino acid sequencing (such as Edman degradation) may be used, as may mass spectrometry (in particular MALDI or ESI techniques (see, e.g., Langen et al. (1997) *Electrophoresis* 18: 1184-1192)). The protein sequences provided herein can be used for the identification of *C. glutamicum* proteins by these techniques.

The information obtained by these methods can be used to compare patterns of protein presence, activity, or modification between different samples from various biological conditions (e.g., different organisms, time points of fermentation, media conditions, or different biotopes, among others). Data obtained

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from such experiments alone, or in combination with other techniques, can be used for various applications, such as to compare the behavior of various organisms in a given (e.g., metabolic) situation, to increase the productivity of strains
5 which produce fine chemicals or to increase the efficiency of the production of fine chemicals.

Equivalents

10 Those of ordinary skill in the art will recognize, or will be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

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Claims

1. An isolated *Corynebacterium glutamicum* nucleic acid molecule
5 selected from the group consisting of those sequences set forth as odd-numbered SEQ ID NOs of the Sequence Listing, or a portion thereof, as set forth in Table 1.
2. An isolated nucleic acid molecule which encodes a polypeptide sequence selected from the group consisting of those
10 sequences set forth as even-numbered SEQ ID NOs of the Sequence Listing, as set forth in Table 1.
3. An isolated nucleic acid molecule which encodes a naturally occurring allelic variant of a polypeptide selected from the
15 group of amino acid sequences consisting of those sequences set forth as even-numbered SEQ ID NOs of the Sequence Listing, as set forth in Table 1.
- 20 4. An isolated nucleic acid molecule comprising a nucleotide sequence which is at least 63% homologous on basis of its amino acid sequence to a nucleotide sequence selected from the group consisting of those sequences which encode for an amino acid sequence as set forth as SEQ ID NO 2 of the
25 Sequence Listing, or a portion thereof, or sequence which is at least 71% homologous on basis of its amino acid sequence to a nucleotide sequence selected from the group consisting of those sequences which encode for an amino acid sequence as set forth as SEQ ID NO 4 of the Sequence Listing, or a
30 portion thereof.
5. An isolated nucleic acid molecule comprising a fragment of at least 15 nucleotides of a nucleic acid comprising a
35 nucleotide sequence selected from the group consisting of those sequences set forth as odd-numbered SEQ ID NOs of the Sequence Listing, as set forth in Table 1.
6. An isolated nucleic acid molecule which hybridizes to the nucleic acid molecule of any one of claims 1-5 under
40 stringent conditions.
7. An isolated nucleic acid molecule comprising the nucleic acid molecule of any one of claims 1-6 or a portion thereof and a
45 nucleotide sequence encoding a heterologous polypeptide.

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8. A DNA-construct comprising the nucleic acid molecule of any one of claims 1-7 and a regulatory sequence.
9. A vector comprising the nucleic acid molecule of any one of claims 1-7.
10. A vector of claim 9 comprising in addition one or more copies of the same or different nucleic acid molecule of table 4 provided the nucleic acid molecule pertains methionine or of table 5 provided the nucleic acid molecule pertains trehalose.
11. The vector of any one of the claims 9 or 10, which is an expression vector.
12. A host cell transfected with the expression vector of claim 11.
13. The host cell of claim 12, wherein said cell is a micro-organism.
14. The host cell of claim 13, wherein said cell belongs to the genus *Corynebacterium* or *Brevibacterium*.
15. The host cell of claim 12, wherein the expression of said nucleic acid molecule results in the modulation in production of a fine chemical from said cell.
16. The host cell of claim 15, wherein said fine chemical is selected from the group consisting of: organic acids, non-proteinogenic amino acids, purine and pyrimidine bases, nucleosides, nucleotides, lipids, saturated and unsaturated fatty acids, diols, carbohydrates, aromatic compounds, vitamins, cofactors, polyketides, and enzymes.
17. A method of producing a polypeptide comprising culturing the host cell of claim 12 in an appropriate culture medium to, thereby, produce the polypeptide.
18. An isolated polypeptide comprising an amino acid sequence selected from the group consisting of those sequences set forth as even-numbered SEQ ID NOs of the Sequence Listing, as set forth in Table 1.

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19. An isolated polypeptide comprising a naturally occurring allelic variant of a polypeptide comprising an amino acid sequence selected from the group consisting of those sequences set forth as even-numbered SEQ ID NOs of the Sequence Listing, or a portion thereof, as set forth in Table 1.
20. The isolated polypeptide of any of claims 18 or 19, further comprising heterologous amino acid sequences.
21. An isolated polypeptide which is encoded by a nucleic acid molecule comprising a nucleotide sequence which is at least 63% homologous to a nucleic acid selected from the group consisting of those sequences set forth as odd-numbered SEQ ID NOs of the Sequence Listing, as set forth in Table 1.
22. An isolated polypeptide comprising an amino acid sequence which is at least 63% homologous to an amino acid sequence selected from the group consisting of those sequences set forth as even-numbered SEQ ID NOs of the Sequence Listing, as set forth in Table 1.
23. A method for producing a fine chemical, comprising culturing a cell containing a vector of claim 11 such that the fine chemical is produced.
24. The method of claim 23, wherein said method further comprises the step of recovering the fine chemical from said culture.
25. The method of claim 23, wherein said method further comprises the step of transfecting said cell with the vector of claim 11 to result in a cell containing said vector.
26. The method of claim 23, wherein said cell belongs to the genus *Corynebacterium* or *Brevibacterium*.
27. The method of claim 23, wherein said cell is selected from the group consisting of: *Corynebacterium glutamicum*, *Corynebacterium herculis*, *Corynebacterium lilium*, *Corynebacterium acetoacidophilum*, *Corynebacterium acetoglutamicum*, *Corynebacterium acetophilum*, *Corynebacterium ammoniagenes*, *Corynebacterium fujiokense*, *Corynebacterium nitrilophilus*, *Brevibacterium ammoniagenes*, *Brevibacterium butanicum*, *Brevibacterium divaricatum*, *Brevibacterium flavum*, *Brevibacterium healii*, *Brevibacterium ketoglutamicum*, *Brevibacterium ketosoreductum*, *Brevibacterium lactofermentum*, *Brevibacterium*

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linens, *Brevibacterium paraffinolyticum*, and those strains set forth in Table 2.

28. The method of claim 23, wherein expression of the nucleic acid molecule from said vector results in modulation of production of said fine chemical.
29. The method of claim 23, wherein said fine chemical is selected from the group consisting of: organic acids, non-proteinogenic amino acids, purine and pyrimidine bases, nucleosides, nucleotides, lipids, saturated and unsaturated fatty acids, diols, carbohydrates, aromatic compounds, vitamins, cofactors, polyketides, and enzymes.
30. The method of claim 23, wherein said fine chemical is an amino acid or a carbohydrate.
31. The method of claim 30, wherein said amino acid carbohydrate is drawn from the group consisting of: methionine or trehalose.
32. A method for producing a fine chemical, comprising culturing a cell whose genomic DNA has been altered by the inclusion of a nucleic acid molecule of any one of claims 1-7.
33. A method for producing a fine chemical of claim 32 comprising in addition one or more copies of the same or different nucleic acid molecule of table 4 provided the nucleic acid molecule pertains methionine or of table 5 provided the nucleic acid molecule pertains trehalose.
34. A method for diagnosing the presence or activity of *Corynebacterium diphtheriae* in a subject, comprising detecting the presence of one or more of SEQ ID NOs 1 through 4 of the Sequence Listing in the subject, thereby diagnosing the presence or activity of *Corynebacterium diphtheriae* in the subject.
35. A host cell comprising a nucleic acid molecule selected from the group consisting of the nucleic acid molecules set forth as odd-numbered SEQ ID NOs of the Sequence Listing, wherein the nucleic acid molecule is disrupted.

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36. A host cell comprising a nucleic acid molecule selected from the group consisting of the nucleic acid molecules set forth as odd-numbered SEQ ID NOs in the Sequence Listing, wherein the nucleic acid molecule comprises one or more nucleic acid modifications from the sequence set forth as odd-numbered SEQ ID NOs of the Sequence Listing.

37. A host cell comprising a nucleic acid molecule selected from the group consisting of the nucleic acid molecules set forth as odd-numbered SEQ ID NOs of the Sequence Listing, wherein the regulatory region of the nucleic acid molecule is modified relative to the wild-type regulatory region of the molecule.

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SEQUENZPROTOKOLL

<110> BASF Aktiengesellschaft

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                                         1           5

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Thr Ser Pro Ala His Asn Asn Ala His Ser Ser Glu Phe Leu Asp Ala
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2

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3

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4

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Ala Glu Arg Met Arg Phe Ser Phe Pro Arg Gln Gln Arg Gly Arg Phe	
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ttg tgc atc gcg gat ttc att cgc cca cgc gag caa gct gtc aag gac	3331
Leu Cys Ile Ala Asp Phe Ile Arg Pro Arg Glu Gln Ala Val Lys Asp	
1065 1070 1075	
ggc caa gtg gac gtc atg cca ttc cag ctg gtc acc atg ggt aat cct	3379
Gly Gln Val Asp Val Met Pro Phe Gln Leu Val Thr Met Gly Asn Pro	
1080 1085 1090	
att gct gat ttc gcc aac gag ttg ttc gca gcc aat gaa tac cgc gag	3427
Ile Ala Asp Phe Ala Asn Glu Leu Phe Ala Ala Asn Glu Tyr Arg Glu	
1095 1100 1105	
tac ttg gaa gtt cac ggc atc ggc gtg cag ctc acc gaa gca ttg gcc	3475
Tyr Leu Glu Val His Gly Ile Gly Val Gln Leu Thr Glu Ala Leu Ala	
1110 1115 1120 1125	
gag tac tgg cac tcc cga gtg cgc agc gaa ctc aag ctg aac gac ggt	3523
Glu Tyr Trp His Ser Arg Val Arg Ser Glu Leu Lys Leu Asn Asp Gly	
1130 1135 1140	

7

gga tct gtc gct gat ttt gat cca gaa gac aag acc aag ttc ttc gac 3571
 Gly Ser Val Ala Asp Phe Asp Pro Glu Asp Lys Thr Lys Phe Phe Asp
 1145 1150 1155
 ctg gat tac cgc ggc gcc cgc ttc tcc ttt ggt tac ggt tct tgc cct 3619
 Leu Asp Tyr Arg Gly Ala Arg Phe Ser Phe Gly Tyr Gly Ser Cys Pro
 1160 1165 1170
 gat ctg gaa gac cgc gca aag ctg gtg gaa ttg ctc gag cca ggc cgt 3667
 Asp Leu Glu Asp Arg Ala Lys Leu Val Glu Leu Leu Glu Pro Gly Arg
 1175 1180 1185
 atc ggc gtg gag ttg tcc gag gaa ctc cag ctg cac cca gag cag tcc 3715
 Ile Gly Val Glu Leu Ser Glu Glu Leu Gln Leu His Pro Glu Gln Ser
 1190 1195 1200 1205
 aca gac gcg ttt gtg ctc tac cac cca gag gca aag tac ttt aac gtc 3763
 Thr Asp Ala Phe Val Leu Tyr His Pro Glu Ala Lys Tyr Phe Asn Val
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 taacaccttt gagaggaaa actttcccg 3793

<210> 2

<211> 1221

<212> PRT

<213> Corynebacterium glutamicum

<400> 2

Met Ser Thr Ser Val Thr Ser Pro Ala His Asn Asn Ala His Ser Ser
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 Glu Phe Leu Asp Ala Leu Ala Asn His Val Leu Ile Gly Asp Gly Ala
 20 25 30
 Met Gly Thr Gln Leu Gln Gly Phe Asp Leu Asp Val Glu Lys Asp Phe
 35 40 45
 Leu Asp Leu Glu Gly Cys Asn Glu Ile Leu Asn Asp Thr Arg Pro Asp
 50 55 60
 Val Leu Arg Gln Ile His Arg Ala Tyr Phe Glu Ala Gly Ala Asp Leu
 65 70 75 80
 Val Glu Thr Asn Thr Phe Gly Cys Asn Leu Pro Asn Leu Ala Asp Tyr
 85 90 95
 Asp Ile Ala Asp Arg Cys Arg Glu Leu Ala Tyr Lys Gly Thr Ala Val
 100 105 110
 Ala Arg Glu Val Ala Asp Glu Met Gly Pro Gly Arg Asn Gly Met Arg
 115 120 125
 Arg Phe Val Val Gly Ser Leu Gly Pro Gly Thr Lys Leu Pro Ser Leu
 130 135 140

8

Gly His Ala Pro Tyr Ala Asp Leu Arg Gly His Tyr Lys Glu Ala Ala			
145	150	155	160
Leu Gly Ile Ile Asp Gly Gly Gly Asp Ala Phe Leu Ile Glu Thr Ala			
	165	170	175
Gln Asp Leu Leu Gln Val Lys Ala Ala Val His Gly Val Gln Asp Ala			
	180	185	190
Met Ala Glu Leu Asp Thr Phe Leu Pro Ile Ile Cys His Val Thr Val			
	195	200	205
Glu Thr Thr Gly Thr Met Leu Met Gly Ser Glu Ile Gly Ala Ala Leu			
	210	215	220
Thr Ala Leu Gln Pro Leu Gly Ile Asp Met Ile Gly Leu Asn Cys Ala			
	225	230	235
Thr Gly Pro Asp Glu Met Ser Glu His Leu Arg Tyr Leu Ser Lys His			
	245	250	255
Ala Asp Ile Pro Val Ser Val Met Pro Asn Ala Gly Leu Pro Val Leu			
	260	265	270
Gly Lys Asn Gly Ala Glu Tyr Pro Leu Glu Ala Glu Asp Leu Ala Gln			
	275	280	285
Ala Leu Ala Gly Phe Val Ser Glu Tyr Gly Leu Ser Met Val Gly Gly			
	290	295	300
Cys Cys Gly Thr Thr Pro Glu His Ile Arg Ala Val Arg Asp Ala Val			
	305	310	315
Val Gly Val Pro Glu Gln Glu Thr Ser Thr Leu Thr Lys Ile Pro Ala			
	325	330	335
Gly Pro Val Glu Gln Ala Ser Arg Glu Val Glu Lys Glu Asp Ser Val			
	340	345	350
Ala Ser Leu Tyr Thr Ser Val Pro Leu Ser Gln Glu Thr Gly Ile Ser			
	355	360	365
Met Ile Gly Glu Arg Thr Asn Ser Asn Gly Ser Lys Ala Phe Arg Glu			
	370	375	380
Ala Met Leu Ser Gly Asp Trp Glu Lys Cys Val Asp Ile Ala Lys Gln			
	385	390	395
Gln Thr Arg Asp Gly Ala His Met Leu Asp Leu Cys Val Asp Tyr Val			
	405	410	415
Gly Arg Asp Gly Thr Ala Asp Met Ala Thr Leu Ala Ala Leu Leu Ala			
	420	425	430
Thr Ser Ser Thr Leu Pro Ile Met Ile Asp Ser Thr Glu Pro Glu Val			
	435	440	445

9

Ile Arg Thr Gly Leu Glu His Leu Gly Gly Arg Ser Ile Val Asn Ser
 450 455 460
 Val Asn Phe Glu Asp Gly Asp Gly Pro Glu Ser Arg Tyr Gln Arg Ile
 465 470 475 480
 Met Lys Leu Val Lys Gln His Gly Ala Ala Val Val Ala Leu Thr Ile
 485 490 495
 Asp Glu Glu Gly Gln Ala Arg Thr Ala Glu His Lys Val Arg Ile Ala
 500 505 510
 Lys Arg Leu Ile Asp Asp Ile Thr Gly Ser Tyr Gly Leu Asp Ile Lys
 515 520 525
 Asp Ile Val Val Asp Cys Leu Thr Phe Pro Ile Ser Thr Gly Gln Glu
 530 535 540
 Glu Thr Arg Arg Asp Gly Ile Glu Thr Ile Glu Ala Ile Arg Glu Leu
 545 550 555 560
 Lys Lys Leu Tyr Pro Glu Ile His Thr Thr Leu Gly Leu Ser Asn Ile
 565 570 575
 Ser Phe Gly Leu Asn Pro Ala Ala Arg Gln Val Leu Asn Ser Val Phe
 580 585 590
 Leu Asn Glu Cys Ile Glu Ala Gly Leu Asp Ser Ala Ile Ala His Ser
 595 600 605
 Ser Lys Ile Leu Pro Met Asn Arg Ile Asp Asp Arg Gln Arg Glu Val
 610 615 620
 Ala Leu Asp Met Val Tyr Asp Arg Arg Thr Glu Asp Tyr Asp Pro Leu
 625 630 635 640
 Gln Glu Phe Met Gln Leu Phe Glu Gly Val Ser Ala Ala Asp Ala Lys
 645 650 655
 Asp Ala Arg Ala Glu Gln Leu Ala Ala Met Pro Leu Phe Glu Arg Leu
 660 665 670
 Ala Gln Arg Ile Ile Asp Gly Asp Lys Asn Gly Leu Glu Asp Asp Leu
 675 680 685
 Glu Ala Gly Met Lys Glu Lys Ser Pro Ile Ala Ile Ile Asn Glu Asp
 690 695 700
 Leu Leu Asn Gly Met Lys Thr Val Gly Glu Leu Phe Gly Ser Gly Gln
 705 710 715 720
 Met Gln Leu Pro Phe Val Leu Gln Ser Ala Glu Thr Met Lys Thr Ala
 725 730 735
 Val Ala Tyr Leu Glu Pro Phe Met Glu Glu Glu Ala Glu Ala Thr Gly
 740 745 750

10

Ser Ala Gln Ala Glu Gly Lys Gly Lys Ile Val Val Ala Thr Val Lys
 755 760 765
 Gly Asp Val His Asp Ile Gly Lys Asn Leu Val Asp Ile Ile Leu Ser
 770 775 780
 Asn Asn Gly Tyr Asp Val Val Asn Leu Gly Ile Lys Gln Pro Leu Ser
 785 790 795 800
 Ala Met Leu Glu Ala Ala Glu Glu His Lys Ala Asp Val Ile Gly Met
 805 810 815
 Ser Gly Leu Leu Val Lys Ser Thr Val Val Met Lys Glu Asn Leu Glu
 820 825 830
 Glu Xaa Asn Asn Ala Gly Ala Ser Asn Tyr Pro Val Ile Leu Gly Gly
 835 840 845
 Ala Ala Leu Thr Arg Thr Tyr Val Glu Asn Asp Leu Asn Glu Val Tyr
 850 855 860
 Thr Gly Glu Val Tyr Tyr Ala Arg Asp Ala Phe Glu Gly Leu Arg Leu
 865 870 875 880
 Met Asp Glu Val Met Ala Glu Lys Arg Gly Glu Gly Leu Asp Pro Asn
 885 890 895
 Ser Pro Glu Ala Ile Glu Gln Ala Lys Lys Lys Ala Glu Arg Lys Ala
 900 905 910
 Arg Asn Glu Arg Ser Arg Lys Ile Ala Ala Glu Arg Lys Ala Asn Ala
 915 920 925
 Ala Pro Val Ile Val Pro Glu Arg Ser Asp Val Ser Thr Asp Thr Pro
 930 935 940
 Thr Ala Ala Pro Pro Phe Trp Gly Thr Arg Ile Val Lys Gly Leu Pro
 945 950 955 960
 Leu Ala Glu Phe Leu Gly Asn Leu Asp Glu Arg Ala Leu Phe Met Gly
 965 970 975
 Gln Trp Gly Leu Lys Ser Thr Arg Gly Asn Glu Gly Pro Ser Tyr Glu
 980 985 990
 Asp Leu Val Glu Thr Glu Gly Arg Pro Arg Leu Arg Tyr Trp Leu Asp
 995 1000 1005
 Arg Leu Lys Ser Glu Gly Ile Leu Asp His Val Ala Leu Val Tyr Gly
 1010 1015 1020
 Tyr Phe Pro Ala Val Ala Glu Gly Asp Asp Val Val Ile Leu Glu Ser
 1025 1030 1035 1040
 Pro Asp Pro His Ala Ala Glu Arg Met Arg Phe Ser Phe Pro Arg Gln
 1045 1050 1055

11

Gln Arg Gly Arg Phe Leu Cys Ile Ala Asp Phe Ile Arg Pro Arg Glu
 1060 1065 1070

Gln Ala Val Lys Asp Gly Gln Val Asp Val Met Pro Phe Gln Leu Val
 1075 1080 1085

Thr Met Gly Asn Pro Ile Ala Asp Phe Ala Asn Glu Leu Phe Ala Ala
 1090 1095 1100

Asn Glu Tyr Arg Glu Tyr Leu Glu Val His Gly Ile Gly Val Gln Leu
 1105 1110 1115 1120

Thr Glu Ala Leu Ala Glu Tyr Trp His Ser Arg Val Arg Ser Glu Leu
 1125 1130 1135

Lys Leu Asn Asp Gly Gly Ser Val Ala Asp Phe Asp Pro Glu Asp Lys
 1140 1145 1150

Thr Lys Phe Phe Asp Leu Asp Tyr Arg Gly Ala Arg Phe Ser Phe Gly
 1155 1160 1165

Tyr Gly Ser Cys Pro Asp Leu Glu Asp Arg Ala Lys Leu Val Glu Leu
 1170 1175 1180

Leu Glu Pro Gly Arg Ile Gly Val Glu Leu Ser Glu Glu Leu Gln Leu
 1185 1190 1195 1200

His Pro Glu Gln Ser Thr Asp Ala Phe Val Leu Tyr His Pro Glu Ala
 1205 1210 1215

Lys Tyr Phe Asn Val
 1220

<210> 3

<211> 1981

<212> DNA

<213> Corynebacterium glutamicum

<220>

<221> CDS

<222> (101)..(1951)

<400> 3

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gaaagacccg ctacgcatgg tgcgccctggc tttttagaat gtg ctg caa acc tcc 115
 Val Leu Gln Thr Ser
 1 5

tgg cat ttc tct atc ctg gca ggc atg act gat acc tct ccg ttg aat 163
 Trp His Phe Ser Ile Leu Ala Gly Met Thr Asp Thr Ser Pro Leu Asn
 10 15 20

12

tct cag ccg agt gca gat cac cac cct gat cac gcg gct cgc cca gtt	211
Ser Gln Pro Ser Ala Asp His His Pro Asp His Ala Ala Arg Pro Val	
25 30 35	
ctt gat gcc cac ggc ttg atc gtt gag cac gaa tcg gaa gag ttt cca	259
Leu Asp Ala His Gly Leu Ile Val Glu His Glu Ser Glu Glu Phe Pro	
40 45 50	
gtc ccc gca ccc gct ccc ggt gaa cag ccc tgg gag aag aaa aac cgc	307
Val Pro Ala Pro Ala Pro Gly Glu Gln Pro Trp Glu Lys Lys Asn Arg	
55 60 65	
gag tgg tac aaa gac gcc gtt ttc tac gaa gtg ctg gtt cgt gcc ttc	355
Glu Trp Tyr Lys Asp Ala Val Phe Tyr Glu Val Leu Val Arg Ala Phe	
70 75 80 85	
tac gat cca gaa ggc aac gga gtc gga tcg ttg aaa ggc ctg acc gaa	403
Tyr Asp Pro Glu Gly Asn Gly Val Gly Ser Leu Lys Gly Leu Thr Glu	
90 95 100	
aaa ctg gat tac atc cag tgg ctc ggc gtg gat tgc att tgg atc cca	451
Lys Leu Asp Tyr Ile Gln Trp Leu Gly Val Asp Cys Ile Trp Ile Pro	
105 110 115	
ccg ttt tat gat tcc cca ctg cgc gac ggc ggt tac gat atc cgc aac	499
Pro Phe Tyr Asp Ser Pro Leu Arg Asp Gly Gly Tyr Asp Ile Arg Asn	
120 125 130	
ttc cgt gaa atc ctg ccc gaa ttc ggc acc gtc gat gac ttc gtg gaa	547
Phe Arg Glu Ile Leu Pro Glu Phe Gly Thr Val Asp Asp Phe Val Glu	
135 140 145	
ctc gtt gac cac gcc cac cgc cgt ggc ctg cgt gtt atc acc gac ttg	595
Leu Val Asp His Ala His Arg Arg Gly Leu Arg Val Ile Thr Asp Leu	
150 155 160 165	
gtc atg aat cac acc tcc gac cag cac gca tgg ttc caa gaa tcc cgg	643
Val Met Asn His Thr Ser Asp Gln His Ala Trp Phe Gln Glu Ser Arg	
170 175 180	
cgc gac cca acc ggc ccc tac gga gat ttc tat gtg tgg agc gat gat	691
Arg Asp Pro Thr Gly Pro Tyr Gly Asp Phe Tyr Val Trp Ser Asp Asp	
185 190 195	
ccc acc ctg tac aac gaa gcc cgc atc atc ttt gta gat aca gaa gaa	739
Pro Thr Leu Tyr Asn Glu Ala Arg Ile Ile Phe Val Asp Thr Glu Glu	
200 205 210	
tcc aac tgg acc tat gat ccg gtg cgt ggc cag tac ttc tgg cac cgc	787
Ser Asn Trp Thr Tyr Asp Pro Val Arg Gly Gln Tyr Phe Trp His Arg	
215 220 225	

13

ttc ttc tcc cac caa cca gac ctc aac tac gac aac ccc gca gtc caa	835
Phe Phe Ser His Gln Pro Asp Leu Asn Tyr Asp Asn Pro Ala Val Gln	
230 235 240 245	
gag gcc atg cta gat gtc ttg cgt ttc tgg ctg gac ctg gga ctt gat	883
Glu Ala Met Leu Asp Val Leu Arg Phe Trp Leu Asp Leu Gly Leu Asp	
250 255 260	
ggt ttc cga cta gat gcc gtt cct tat ctt ttt gaa cgc gaa ggc acc	931
Gly Phe Arg Leu Asp Ala Val Pro Tyr Leu Phe Glu Arg Glu Gly Thr	
265 270 275	
aac ggc gaa aac ctc aaa gaa acc cac gat ttc ctc aaa ctg tgt cgc	979
Asn Gly Glu Asn Leu Lys Glu Thr His Asp Phe Leu Lys Leu Cys Arg	
280 285 290	
tct gtc att gag aag gaa tac ccc ggc cga atc ctg ctc gca gaa gcc	1027
Ser Val Ile Glu Lys Glu Tyr Pro Gly Arg Ile Leu Leu Ala Glu Ala	
295 300 305	
aac caa tgg ccc caa gat gtg gtc gaa tac ttc ggt gaa aaa gac aaa	1075
Asn Gln Trp Pro Gln Asp Val Val Glu Tyr Phe Gly Glu Lys Asp Lys	
310 315 320 325	
ggc gat gaa tgc cac atg gcc ttc cac ttc cct ttg atg ccg cgc atc	1123
Gly Asp Glu Cys His Met Ala Phe His Phe Pro Leu Met Pro Arg Ile	
330 335 340	
ttc atg gga gtt cgc caa ggt tca cgc acc ccg atc agt gag atc ctg	1171
Phe Met Gly Val Arg Gln Gly Ser Arg Thr Pro Ile Ser Glu Ile Leu	
345 350 355	
gcc aac acc ccg gag att ccc aag act gcc caa tgg ggt att ttc ctg	1219
Ala Asn Thr Pro Glu Ile Pro Lys Thr Ala Gln Trp Gly Ile Phe Leu	
360 365 370	
cgt aat cat gat gag ctc acc ctt gaa atg gtc tcc gat gag gaa cgc	1267
Arg Asn His Asp Glu Leu Thr Leu Glu Met Val Ser Asp Glu Glu Arg	
375 380 385	
agc tac atg tac tcc caa ttc gcc tcc gaa cct cgc atg cgc gcc aac	1315
Ser Tyr Met Tyr Ser Gln Phe Ala Ser Glu Pro Arg Met Arg Ala Asn	
390 395 400 405	
gta gga atc cgc agg cgc ctt tcc cca ctg ctt gaa ggc gac cgc aac	1363
Val Gly Ile Arg Arg Arg Leu Ser Pro Leu Leu Glu Gly Asp Arg Asn	
410 415 420	
cag ctg gaa ctc ctt cac ggt ttg ttg ctg tct cta cct ggc tca ccc	1411
Gln Leu Glu Leu Leu His Gly Leu Leu Leu Ser Leu Pro Gly Ser Pro	
425 430 435	

14

gtg ttg tat tac ggt gat gaa att ggc atg ggc gac aat atc tgg ctc	1459
Val Leu Tyr Tyr Gly Asp Glu Ile Gly Met Gly Asp Asn Ile Trp Leu	
440 445 450	
cac gac cgc gac gga gtg cgc acc ccc atg cag tgg tcc aac gac cgc	1507
His Asp Arg Asp Gly Val Arg Thr Pro Met Gln Trp Ser Asn Asp Arg	
455 460 465	
aac ggt ggt ttc tcc aaa gct gat cct gaa cgc ctg tac ctt cca gcg	1555
Asn Gly Gly Phe Ser Lys Ala Asp Pro Glu Arg Leu Tyr Leu Pro Ala	
470 475 480 485	
atc caa aat gat caa tac ggc tac gcc caa gta aac gtg gaa agc caa	1603
Ile Gln Asn Asp Gln Tyr Gly Tyr Ala Gln Val Asn Val Glu Ser Gln	
490 495 500	
ctc aac cgc gaa aac tcc ctg ctg cgc tgg ctc cga aac caa atc ctt	1651
Leu Asn Arg Glu Asn Ser Leu Leu Arg Trp Leu Arg Asn Gln Ile Leu	
505 510 515	
atc cgc aag cag tac cgc gca ttt ggt gcc gga acc tac cgt gaa gtg	1699
Ile Arg Lys Gln Tyr Arg Ala Phe Gly Ala Gly Thr Tyr Arg Glu Val	
520 525 530	
tcc tcc acc aat gag tca gtg ttg aca ttt tta cga gaa cac aag ggc	1747
Ser Ser Thr Asn Glu Ser Val Leu Thr Phe Leu Arg Glu His Lys Gly	
535 540 545	
caa acc att ttg tgt gtc aac aac atg agc aaa tat cct cag gca gtc	1795
Gln Thr Ile Leu Cys Val Asn Asn Met Ser Lys Tyr Pro Gln Ala Val	
550 555 560 565	
tcg ctt gat ttg cgt gaa ttt gca gga cac acc cct cga gag atg tcg	1843
Ser Leu Asp Leu Arg Glu Phe Ala Gly His Thr Pro Arg Glu Met Ser	
570 575 580	
ggc ggg cag ctg ttc cct acc att gct gaa cgg gag tgg att gtc act	1891
Gly Gly Gln Leu Phe Pro Thr Ile Ala Glu Arg Glu Trp Ile Val Thr	
585 590 595	
tta gcc cct cac gga ttc ttc tgg ttt gat ctc acc gcc gat gaa aag	1939
Leu Ala Pro His Gly Phe Phe Trp Phe Asp Leu Thr Ala Asp Glu Lys	
600 605 610	
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Asp Asp Met Glu	
615	

<210> 4

<211> 617

<212> PRT

<213> Corynebacterium glutamicum

15

<400> 4

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			20					25					30		
Ala	Ala	Arg	Pro	Val	Leu	Asp	Ala	His	Gly	Leu	Ile	Val	Glu	His	Glu
		35					40					45			
Ser	Glu	Glu	Phe	Pro	Val	Pro	Ala	Pro	Ala	Pro	Gly	Glu	Gln	Pro	Trp
	50					55					60				
Glu	Lys	Lys	Asn	Arg	Glu	Trp	Tyr	Lys	Asp	Ala	Val	Phe	Tyr	Glu	Val
65					70					75					80
Leu	Val	Arg	Ala	Phe	Tyr	Asp	Pro	Glu	Gly	Asn	Gly	Val	Gly	Ser	Leu
				85					90					95	
Lys	Gly	Leu	Thr	Glu	Lys	Leu	Asp	Tyr	Ile	Gln	Trp	Leu	Gly	Val	Asp
			100					105					110		
Cys	Ile	Trp	Ile	Pro	Pro	Phe	Tyr	Asp	Ser	Pro	Leu	Arg	Asp	Gly	Gly
		115					120					125			
Tyr	Asp	Ile	Arg	Asn	Phe	Arg	Glu	Ile	Leu	Pro	Glu	Phe	Gly	Thr	Val
	130					135					140				
Asp	Asp	Phe	Val	Glu	Leu	Val	Asp	His	Ala	His	Arg	Arg	Gly	Leu	Arg
145					150					155					160
Val	Ile	Thr	Asp	Leu	Val	Met	Asn	His	Thr	Ser	Asp	Gln	His	Ala	Trp
				165					170					175	
Phe	Gln	Glu	Ser	Arg	Arg	Asp	Pro	Thr	Gly	Pro	Tyr	Gly	Asp	Phe	Tyr
			180					185					190		
Val	Trp	Ser	Asp	Asp	Pro	Thr	Leu	Tyr	Asn	Glu	Ala	Arg	Ile	Ile	Phe
		195					200					205			
Val	Asp	Thr	Glu	Glu	Ser	Asn	Trp	Thr	Tyr	Asp	Pro	Val	Arg	Gly	Gln
	210					215					220				
Tyr	Phe	Trp	His	Arg	Phe	Phe	Ser	His	Gln	Pro	Asp	Leu	Asn	Tyr	Asp
225					230					235				240	
Asn	Pro	Ala	Val	Gln	Glu	Ala	Met	Leu	Asp	Val	Leu	Arg	Phe	Trp	Leu
				245					250					255	
Asp	Leu	Gly	Leu	Asp	Gly	Phe	Arg	Leu	Asp	Ala	Val	Pro	Tyr	Leu	Phe
			260					265					270		
Glu	Arg	Glu	Gly	Thr	Asn	Gly	Glu	Asn	Leu	Lys	Glu	Thr	His	Asp	Phe
			275				280							285	

16

Leu Lys Leu Cys Arg Ser Val Ile Glu Lys Glu Tyr Pro Gly Arg Ile
 290 295 300
 Leu Leu Ala Glu Ala Asn Gln Trp Pro Gln Asp Val Val Glu Tyr Phe
 305 310 315 320
 Gly Glu Lys Asp Lys Gly Asp Glu Cys His Met Ala Phe His Phe Pro
 325 330 335
 Leu Met Pro Arg Ile Phe Met Gly Val Arg Gln Gly Ser Arg Thr Pro
 340 345 350
 Ile Ser Glu Ile Leu Ala Asn Thr Pro Glu Ile Pro Lys Thr Ala Gln
 355 360 365
 Trp Gly Ile Phe Leu Arg Asn His Asp Glu Leu Thr Leu Glu Met Val
 370 375 380
 Ser Asp Glu Glu Arg Ser Tyr Met Tyr Ser Gln Phe Ala Ser Glu Pro
 385 390 395 400
 Arg Met Arg Ala Asn Val Gly Ile Arg Arg Arg Leu Ser Pro Leu Leu
 405 410 415
 Glu Gly Asp Arg Asn Gln Leu Glu Leu Leu His Gly Leu Leu Leu Ser
 420 425 430
 Leu Pro Gly Ser Pro Val Leu Tyr Tyr Gly Asp Glu Ile Gly Met Gly
 435 440 445
 Asp Asn Ile Trp Leu His Asp Arg Asp Gly Val Arg Thr Pro Met Gln
 450 455 460
 Trp Ser Asn Asp Arg Asn Gly Gly Phe Ser Lys Ala Asp Pro Glu Arg
 465 470 475 480
 Leu Tyr Leu Pro Ala Ile Gln Asn Asp Gln Tyr Gly Tyr Ala Gln Val
 485 490 495
 Asn Val Glu Ser Gln Leu Asn Arg Glu Asn Ser Leu Leu Arg Trp Leu
 500 505 510
 Arg Asn Gln Ile Leu Ile Arg Lys Gln Tyr Arg Ala Phe Gly Ala Gly
 515 520 525
 Thr Tyr Arg Glu Val Ser Ser Thr Asn Glu Ser Val Leu Thr Phe Leu
 530 535 540
 Arg Glu His Lys Gly Gln Thr Ile Leu Cys Val Asn Asn Met Ser Lys
 545 550 555 560
 Tyr Pro Gln Ala Val Ser Leu Asp Leu Arg Glu Phe Ala Gly His Thr
 565 570 575
 Pro Arg Glu Met Ser Gly Gly Gln Leu Phe Pro Thr Ile Ala Glu Arg
 580 585 590

17

Glu Trp Ile Val Thr Leu Ala Pro His Gly Phe Phe Trp Phe Asp Leu
 595 600 605

Thr Ala Asp Glu Lys Asp Asp Met Glu
 610 615

<210> 5

<211> 1840

<212> DNA

<213> *Corynebacterium glutamicum*

<220>

<221> CDS

<222> (363)..(1676)

<400> 5

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aattcttaac aatggaaaag taacattgag agatgattta taccatcctg caccatttag 180

agtggggcta gtcatacccc cataacccta gctgtacgca atcgatttca aatcagttgg 240

aaaaagtcaa gaaaattacc cgagaattaa ttataccac acagtctatt gcaatagacc 300

aagctgttca gtagggtgca tgggagaaga atttcctaataaaaaactcctt aaggacctcc 360

aa atg cca aag tac gac aat tcc aat gct gac cag tgg ggc ttt gaa 407

Met Pro Lys Tyr Asp Asn Ser Asn Ala Asp Gln Trp Gly Phe Glu

1

5

10

15

acc cgc tcc att cac gca ggc cag tca gta gac gca cag acc agc gca 455

Thr Arg Ser Ile His Ala Gly Gln Ser Val Asp Ala Gln Thr Ser Ala

20

25

30

cga aac ctt ccg atc tac caa tcc acc gct ttc gtg ttc gac tcc gct 503

Arg Asn Leu Pro Ile Tyr Gln Ser Thr Ala Phe Val Phe Asp Ser Ala

35

40

45

gag cac gcc aag cag cgt ttc gca ctt gag gat cta ggc cct gtt tac 551

Glu His Ala Lys Gln Arg Phe Ala Leu Glu Asp Leu Gly Pro Val Tyr

50

55

60

tcc cgc ctc acc aac cca acc gtt gag gct ttg gaa aac cgc atc gct 599

Ser Arg Leu Thr Asn Pro Thr Val Glu Ala Leu Glu Asn Arg Ile Ala

65

70

75

tcc ctc gaa ggt ggc gtc cac gct gta gcg ttc tcc tcc gga cag gcc 647

Ser Leu Glu Gly Gly Val His Ala Val Ala Phe Ser Ser Gly Gln Ala

80

85

90

95

18

gca acc acc aac gcc att ttg aac ctg gca gga gcg ggc gac cac atc 695
 Ala Thr Thr Asn Ala Ile Leu Asn Leu Ala Gly Ala Gly Asp His Ile
 100 105 110

gtc acc tcc cca cgc ctc tac ggt ggc acc gag act cta ttc ctt atc 743
 Val Thr Ser Pro Arg Leu Tyr Gly Gly Thr Glu Thr Leu Phe Leu Ile
 115 120 125

act ctt aac cgc ctg ggt atc gat gtt tcc ttc gtg gaa aac ccc gac 791
 Thr Leu Asn Arg Leu Gly Ile Asp Val Ser Phe Val Glu Asn Pro Asp
 130 135 140

gac cct gag tcc tgg cag gca gcc gtt cag cca aac acc aaa gca ttc 839
 Asp Pro Glu Ser Trp Gln Ala Ala Val Gln Pro Asn Thr Lys Ala Phe
 145 150 155

ttc ggc gag act ttc gcc aac cca cag gca gac gtc ctg gat att cct 887
 Phe Gly Glu Thr Phe Ala Asn Pro Gln Ala Asp Val Leu Asp Ile Pro
 160 165 170 175

gcg gtg gct gaa gtt gcg cac cgc aac agc gtt cca ctg atc atc gac 935
 Ala Val Ala Glu Val Ala His Arg Asn Ser Val Pro Leu Ile Ile Asp
 180 185 190

aac acc atc gct acc gca gcg ctc gtg cgc ccg ctc gag ctc ggc gca 983
 Asn Thr Ile Ala Thr Ala Ala Leu Val Arg Pro Leu Glu Leu Gly Ala
 195 200 205

gac gtt gtc gtc gct tcc ctc acc aag ttc tac acc ggc aac ggc tcc 1031
 Asp Val Val Val Ala Ser Leu Thr Lys Phe Tyr Thr Gly Asn Gly Ser
 210 215 220

gga ctg ggc ggc gtg ctt atc gac ggc gga aag ttc gat tgg act gtc 1079
 Gly Leu Gly Gly Val Leu Ile Asp Gly Gly Lys Phe Asp Trp Thr Val
 225 230 235

gaa aag gat gga aag cca gta ttc ccc tac ttc gtc act cca gat gct 1127
 Glu Lys Asp Gly Lys Pro Val Phe Pro Tyr Phe Val Thr Pro Asp Ala
 240 245 250 255

gct tac cac gga ttg aag tac gca gac ctt ggt gca cca gcc ttc ggc 1175
 Ala Tyr His Gly Leu Lys Tyr Ala Asp Leu Gly Ala Pro Ala Phe Gly
 260 265 270

ctc aag gtt cgc gtt ggc ctt cta cgc gac acc ggc tcc acc ctc tcc 1223
 Leu Lys Val Arg Val Gly Leu Leu Arg Asp Thr Gly Ser Thr Leu Ser
 275 280 285

gca ttc aac gca tgg gct gca gtc cag ggc atc gac acc ctt tcc ctg 1271
 Ala Phe Asn Ala Trp Ala Ala Val Gln Gly Ile Asp Thr Leu Ser Leu

19

290	295	300	
cgc ctg gag cgc cac aac gaa aac gcc atc aag gtt gca gaa ttc ctc			1319
Arg Leu Glu Arg His Asn Glu Asn Ala Ile Lys Val Ala Glu Phe Leu			
305	310	315	
aac aac cac gag aag gtg gaa aag gtt aac ttc gca ggc ctg aag gat			1367
Asn Asn His Glu Lys Val Glu Lys Val Asn Phe Ala Gly Leu Lys Asp			
320	325	330	335
tcc cct tgg tac gca acc aag gaa aag ctt ggc ctg aag tac acc ggc			1415
Ser Pro Trp Tyr Ala Thr Lys Glu Lys Leu Gly Leu Lys Tyr Thr Gly			
340	345	350	
tcc gtt ctc acc ttc gag atc aag ggc ggc aag gat gag gct tgg gca			1463
Ser Val Leu Thr Phe Glu Ile Lys Gly Gly Lys Asp Glu Ala Trp Ala			
355	360	365	
ttt atc gac gcc ctg aag cta cac tcc aac ctt gca aac atc ggc gat			1511
Phe Ile Asp Ala Leu Lys Leu His Ser Asn Leu Ala Asn Ile Gly Asp			
370	375	380	
gtt cgc tcc ctc gtt gtt cac cca gca acc acc acc cat tca cag tcc			1559
Val Arg Ser Leu Val Val His Pro Ala Thr Thr Thr His Ser Gln Ser			
385	390	395	
gac gaa gct ggc ctg gca cgc gcg ggc gtt acc cag tcc acc gtc cgc			1607
Asp Glu Ala Gly Leu Ala Arg Ala Gly Val Thr Gln Ser Thr Val Arg			
400	405	410	415
ctg tcc gtt ggc atc gag acc att gat gat atc atc gct gac ctc gaa			1655
Leu Ser Val Gly Ile Glu Thr Ile Asp Asp Ile Ile Ala Asp Leu Glu			
420	425	430	
ggc ggc ttt gct gca atc tag ctttaaataag actcacccca gtgcttaaag			1706
Gly Gly Phe Ala Ala Ile			
435			
cgctggggttt ttctttttca gactcgtgag aatgcaaact agactagaca gagctgtcca			1766
tatacactgg acgaagtttt agtcttgtcc acccagaaca ggcggttatt ttcattgccca			1826
ccctcgcgcc ttca			1840

<210> 6

<211> 437

<212> PRT

<213> Corynebacterium glutamicum

<400> 6

20

Met	Pro	Lys	Tyr	Asp	Asn	Ser	Asn	Ala	Asp	Gln	Trp	Gly	Phe	Glu	Thr	1	5	10	15
Arg	Ser	Ile	His	Ala	Gly	Gln	Ser	Val	Asp	Ala	Gln	Thr	Ser	Ala	Arg	20	25	30	
Asn	Leu	Pro	Ile	Tyr	Gln	Ser	Thr	Ala	Phe	Val	Phe	Asp	Ser	Ala	Glu	35	40	45	
His	Ala	Lys	Gln	Arg	Phe	Ala	Leu	Glu	Asp	Leu	Gly	Pro	Val	Tyr	Ser	50	55	60	
Arg	Leu	Thr	Asn	Pro	Thr	Val	Glu	Ala	Leu	Glu	Asn	Arg	Ile	Ala	Ser	65	70	75	80
Leu	Glu	Gly	Gly	Val	His	Ala	Val	Ala	Phe	Ser	Ser	Gly	Gln	Ala	Ala	85	90	95	
Thr	Thr	Asn	Ala	Ile	Leu	Asn	Leu	Ala	Gly	Ala	Gly	Asp	His	Ile	Val	100	105	110	
Thr	Ser	Pro	Arg	Leu	Tyr	Gly	Gly	Thr	Glu	Thr	Leu	Phe	Leu	Ile	Thr	115	120	125	
Leu	Asn	Arg	Leu	Gly	Ile	Asp	Val	Ser	Phe	Val	Glu	Asn	Pro	Asp	Asp	130	135	140	
Pro	Glu	Ser	Trp	Gln	Ala	Ala	Val	Gln	Pro	Asn	Thr	Lys	Ala	Phe	Phe	145	150	155	160
Gly	Glu	Thr	Phe	Ala	Asn	Pro	Gln	Ala	Asp	Val	Leu	Asp	Ile	Pro	Ala	165	170	175	
Val	Ala	Glu	Val	Ala	His	Arg	Asn	Ser	Val	Pro	Leu	Ile	Ile	Asp	Asn	180	185	190	
Thr	Ile	Ala	Thr	Ala	Ala	Leu	Val	Arg	Pro	Leu	Glu	Leu	Gly	Ala	Asp	195	200	205	
Val	Val	Val	Ala	Ser	Leu	Thr	Lys	Phe	Tyr	Thr	Gly	Asn	Gly	Ser	Gly	210	215	220	
Leu	Gly	Gly	Val	Leu	Ile	Asp	Gly	Gly	Lys	Phe	Asp	Trp	Thr	Val	Glu	225	230	235	240
Lys	Asp	Gly	Lys	Pro	Val	Phe	Pro	Tyr	Phe	Val	Thr	Pro	Asp	Ala	Ala	245	250	255	
Tyr	His	Gly	Leu	Lys	Tyr	Ala	Asp	Leu	Gly	Ala	Pro	Ala	Phe	Gly	Leu	260	265	270	

21

Lys Val Arg Val Gly Leu Leu Arg Asp Thr Gly Ser Thr Leu Ser Ala
 275 280 285

Phe Asn Ala Trp Ala Ala Val Gln Gly Ile Asp Thr Leu Ser Leu Arg
 290 295 300

Leu Glu Arg His Asn Glu Asn Ala Ile Lys Val Ala Glu Phe Leu Asn
 305 310 315 320

Asn His Glu Lys Val Glu Lys Val Asn Phe Ala Gly Leu Lys Asp Ser
 325 330 335

Pro Trp Tyr Ala Thr Lys Glu Lys Leu Gly Leu Lys Tyr Thr Gly Ser
 340 345 350

Val Leu Thr Phe Glu Ile Lys Gly Gly Lys Asp Glu Ala Trp Ala Phe
 355 360 365

Ile Asp Ala Leu Lys Leu His Ser Asn Leu Ala Asn Ile Gly Asp Val
 370 375 380

Arg Ser Leu Val Val His Pro Ala Thr Thr Thr His Ser Gln Ser Asp
 385 390 395 400

Glu Ala Gly Leu Ala Arg Ala Gly Val Thr Gln Ser Thr Val Arg Leu
 405 410 415

Ser Val Gly Ile Glu Thr Ile Asp Asp Ile Ile Ala Asp Leu Glu Gly
 420 425 430

Gly Phe Ala Ala Ile
 435

<210> 7

<211> 1033

<212> DNA

<213> Corynebacterium glutamicum

<220>

<221> CDS

<222> (101)..(1006)

<400> 7

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tgtaacgcag gattcaccaa tcaatgaaag gtcgaccgac atg agc act gaa gac 115
 Met Ser Thr Glu Asp
 1 5

att gtc gtc gta gca gta gat ggc tcg gac gcc tca aaa caa gct gtt 163

22

Ile	Val	Val	Val	Ala	Val	Asp	Gly	Ser	Asp	Ala	Ser	Lys	Gln	Ala	Val		
				10					15					20			
cgg	tgg	gct	gca	aat	acc	gcc	aac	aaa	cgt	ggc	att	cca	ctt	cgc	ttg	211	
Arg	Trp	Ala	Ala	Asn	Thr	Ala	Asn	Lys	Arg	Gly	Ile	Pro	Leu	Arg	Leu		
			25					30					35				
gct	tcc	agc	tac	acc	atg	cct	cag	ttc	ctc	tac	gca	gag	gga	atg	gtt	259	
Ala	Ser	Ser	Tyr	Thr	Met	Pro	Gln	Phe	Leu	Tyr	Ala	Glu	Gly	Met	Val		
			40				45					50					
cca	cca	caa	gag	ctt	ttc	gat	gac	ctc	cag	gcc	gaa	gcc	ctg	gaa	aag	307	
Pro	Pro	Gln	Glu	Leu	Phe	Asp	Asp	Leu	Gln	Ala	Glu	Ala	Leu	Glu	Lys		
		55				60				65							
att	aac	gaa	gcc	cgt	gac	atc	gcc	cat	gag	gta	gcg	cca	gaa	atc	aag	355	
Ile	Asn	Glu	Ala	Arg	Asp	Ile	Ala	His	Glu	Val	Ala	Pro	Glu	Ile	Lys		
70					75				80					85			
atc	ggg	cac	acc	atc	gct	gaa	ggc	agt	ccc	atc	gac	atg	ctg	ttg	gaa	403	
Ile	Gly	His	Thr	Ile	Ala	Glu	Gly	Ser	Pro	Ile	Asp	Met	Leu	Leu	Glu		
				90				95					100				
atg	tct	ccc	gat	gcc	aca	atg	atc	gtc	atg	ggc	tcc	cgc	gga	ctc	ggc	451	
Met	Ser	Pro	Asp	Ala	Thr	Met	Ile	Val	Met	Gly	Ser	Arg	Gly	Leu	Gly		
			105				110						115				
gga	ctc	tcc	gga	atg	gtc	atg	ggc	tcc	gtc	tcc	ggc	gca	gtg	gtc	agc	499	
Gly	Leu	Ser	Gly	Met	Val	Met	Gly	Ser	Val	Ser	Gly	Ala	Val	Val	Ser		
		120					125					130					
cac	gca	aag	tgt	cca	gtc	gtt	gtt	gtc	cgt	gaa	gac	agc	gca	gtc	aac	547	
His	Ala	Lys	Cys	Pro	Val	Val	Val	Val	Arg	Glu	Asp	Ser	Ala	Val	Asn		
		135				140					145						
gaa	gac	agc	aag	tac	ggc	cca	gtc	gtc	gtc	ggc	gtg	gat	ggc	tcc	gaa	595	
Glu	Asp	Ser	Lys	Tyr	Gly	Pro	Val	Val	Val	Gly	Val	Asp	Gly	Ser	Glu		
150					155					160				165			
gtc	tcc	caa	cag	gca	acc	gaa	tac	gca	ttt	gcg	gaa	gct	gaa	gct	cgt	643	
Val	Ser	Gln	Gln	Ala	Thr	Glu	Tyr	Ala	Phe	Ala	Glu	Ala	Glu	Ala	Arg		
				170				175					180				
ggc	gcc	gaa	ctc	gtt	gca	gtt	cac	acc	tgg	atg	gac	atg	cag	gta	cag	691	
Gly	Ala	Glu	Leu	Val	Ala	Val	His	Thr	Trp	Met	Asp	Met	Gln	Val	Gln		
			185				190						195				
gca	tca	ctt	gca	ggc	ctt	gca	gct	gct	caa	cag	cag	tgg	gat	gaa	gtg	739	
Ala	Ser	Leu	Ala	Gly	Leu	Ala	Ala	Ala	Gln	Gln	Gln	Trp	Asp	Glu	Val		
		200					205					210					

23

gaa cgt cag caa acc gac atg ctg atc gaa cgc ctc gca cca ctg gtg 787
 Glu Arg Gln Gln Thr Asp Met Leu Ile Glu Arg Leu Ala Pro Leu Val
 215 220 225

 gaa aag tac cca agt gta acc gtc aag aag atc atc acc cgt gac cgc 835
 Glu Lys Tyr Pro Ser Val Thr Val Lys Lys Ile Ile Thr Arg Asp Arg
 230 235 240 245

 cca gtt cgc gca ctt gca gaa gca tct gaa aac gcg cag ctc cta gtc 883
 Pro Val Arg Ala Leu Ala Glu Ala Ser Glu Asn Ala Gln Leu Leu Val
 250 255 260

 gtt ggt tcc cat ggt cgt ggc gga ttt aag ggc atg ctc ctt ggc tcc 931
 Val Gly Ser His Gly Arg Gly Gly Phe Lys Gly Met Leu Leu Gly Ser
 265 270 275

 acc tcc cgc gca ctg ctg caa tcc gca ccg tgc cca atg atg gtg gtt 979
 Thr Ser Arg Ala Leu Leu Gln Ser Ala Pro Cys Pro Met Met Val Val
 280 285 290

 cgc cca cct gag aag att aag aag tag tttcttttaa gtttcgatgc cccgggtt 1033
 Arg Pro Pro Glu Lys Ile Lys Lys
 295 300

<210> 8

<211> 301

<212> PRT

<213> Corynebacterium glutamicum

<400> 8

Met Ser Thr Glu Asp Ile Val Val Val Ala Val Asp Gly Ser Asp Ala
 1 5 10 15

Ser Lys Gln Ala Val Arg Trp Ala Ala Asn Thr Ala Asn Lys Arg Gly
 20 25 30

Ile Pro Leu Arg Leu Ala Ser Ser Tyr Thr Met Pro Gln Phe Leu Tyr
 35 40 45

Ala Glu Gly Met Val Pro Pro Gln Glu Leu Phe Asp Asp Leu Gln Ala
 50 55 60

Glu Ala Leu Glu Lys Ile Asn Glu Ala Arg Asp Ile Ala His Glu Val
 65 70 75 80

Ala Pro Glu Ile Lys Ile Gly His Thr Ile Ala Glu Gly Ser Pro Ile
 85 90 95

Asp Met Leu Leu Glu Met Ser Pro Asp Ala Thr Met Ile Val Met Gly
 100 105 110

24

Ser Arg Gly Leu Gly Gly Leu Ser Gly Met Val Met Gly Ser Val Ser
 115 120 125
 Gly Ala Val Val Ser His Ala Lys Cys Pro Val Val Val Val Arg Glu
 130 135 140
 Asp Ser Ala Val Asn Glu Asp Ser Lys Tyr Gly Pro Val Val Val Gly
 145 150 155 160
 Val Asp Gly Ser Glu Val Ser Gln Gln Ala Thr Glu Tyr Ala Phe Ala
 165 170 175
 Glu Ala Glu Ala Arg Gly Ala Glu Leu Val Ala Val His Thr Trp Met
 180 185 190
 Asp Met Gln Val Gln Ala Ser Leu Ala Gly Leu Ala Ala Ala Gln Gln
 195 200 205
 Gln Trp Asp Glu Val Glu Arg Gln Gln Thr Asp Met Leu Ile Glu Arg
 210 215 220
 Leu Ala Pro Leu Val Glu Lys Tyr Pro Ser Val Thr Val Lys Lys Ile
 225 230 235 240
 Ile Thr Arg Asp Arg Pro Val Arg Ala Leu Ala Glu Ala Ser Glu Asn
 245 250 255
 Ala Gln Leu Leu Val Val Gly Ser His Gly Arg Gly Gly Phe Lys Gly
 260 265 270
 Met Leu Leu Gly Ser Thr Ser Arg Ala Leu Leu Gln Ser Ala Pro Cys
 275 280 285
 Pro Met Met Val Val Arg Pro Pro Glu Lys Ile Lys Lys
 290 295 300

SEQUENCE LISTING

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<211> 1527

<212> DNA

<213> Corynebacterium glutamicum

<220>

<221> CDS

<222> (101)..(1504)

<223> RXS00315

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25

cgtttcggcg gcaatgagtt cctgggcgcc gcgtattggt atg gcg atg gtg ttc	115
Met Ala Met Val Phe	
1 5	
ccg agc ttg gtg aac ggc tac gac gtg gcc gcc acc atg gct gcg ggc	163
Pro Ser Leu Val Asn Gly Tyr Asp Val Ala Ala Thr Met Ala Ala Gly	
10 15 20	
gaa atg cca atg tgg tcc ctg ttt ggt tta gat gtt gcc caa gcc ggt	211
Glu Met Pro Met Trp Ser Leu Phe Gly Leu Asp Val Ala Gln Ala Gly	
25 30 35	
tac cag ggc acc gtg ctt cct gtg ctg gtg gtt tct tgg att ctg gca	259
Tyr Gln Gly Thr Val Leu Pro Val Leu Val Val Ser Trp Ile Leu Ala	
40 45 50	
acg atc gag aag ttc ctg cac aag cga ctc aag ggc act gca gac ttc	307
Thr Ile Glu Lys Phe Leu His Lys Arg Leu Lys Gly Thr Ala Asp Phe	
55 60 65	
ctg atc act cca gtg ctg acg ttg ctg ctc acc gga ttc ctt aca ttc	355
Leu Ile Thr Pro Val Leu Thr Leu Leu Leu Thr Gly Phe Leu Thr Phe	
70 75 80 85	
atc gcc att ggc cca gca atg cgc tgg gtg ggc gat gtg ctg gca cac	403
Ile Ala Ile Gly Pro Ala Met Arg Trp Val Gly Asp Val Leu Ala His	
90 95 100	
ggt cta cag gga ctt tat gat ttc ggt ggt cca gtc ggc ggt ctg ctc	451
Gly Leu Gln Gly Leu Tyr Asp Phe Gly Gly Pro Val Gly Gly Leu Leu	
105 110 115	
ttc ggt ctg gtc tac tca cca atc gtc atc act ggt ctg cac cag tcc	499
Phe Gly Leu Val Tyr Ser Pro Ile Val Ile Thr Gly Leu His Gln Ser	
120 125 130	
ttc ccg cca att gag ctg gag ctg ttt aac cag ggt gga tcc ttc atc	547
Phe Pro Pro Ile Glu Leu Glu Leu Phe Asn Gln Gly Gly Ser Phe Ile	
135 140 145	
ttc gca acg gca tct atg gct aat atc gcc cag ggt gcg gca tgt ttg	595
Phe Ala Thr Ala Ser Met Ala Asn Ile Ala Gln Gly Ala Ala Cys Leu	
150 155 160 165	
gca gtg ttc ttc ctg gcg aag agt gaa aag ctc aag ggc ctt gca ggt	643
Ala Val Phe Phe Leu Ala Lys Ser Glu Lys Leu Lys Gly Leu Ala Gly	
170 175 180	
gct tca ggt gtc tcc gct gtt ctt ggt att acg gag cct gcg atc ttc	691
Ala Ser Gly Val Ser Ala Val Leu Gly Ile Thr Glu Pro Ala Ile Phe	
185 190 195	

26

ggt gtg aac ctt cgc ctg cgc tgg ccg ttc ttc atc ggt atc ggt acc	739
Gly Val Asn Leu Arg Leu Arg Trp Pro Phe Phe Ile Gly Ile Gly Thr	
200 205 210	
gca gct atc ggt ggc gct ttg att gca ctc ttt aat atc aag gca gtt	787
Ala Ala Ile Gly Gly Ala Leu Ile Ala Leu Phe Asn Ile Lys Ala Val	
215 220 225	
gcg ttg ggc gct gca ggt ttc ttg ggt gtt gtt tct att gat gct cca	835
Ala Leu Gly Ala Ala Gly Phe Leu Gly Val Val Ser Ile Asp Ala Pro	
230 235 240 245	
gat atg gtc atg ttc ttg gtg tgt gca gtt gtt acc ttc ttc atc gca	883
Asp Met Val Met Phe Leu Val Cys Ala Val Val Thr Phe Phe Ile Ala	
250 255 260	
ttc ggc gca gcg att gct tat ggc ctt tac ttg gtt cgc cgc aac ggc	931
Phe Gly Ala Ala Ile Ala Tyr Gly Leu Tyr Leu Val Arg Arg Asn Gly	
265 270 275	
agc att gat cca gat gca acc gct gct cca gtg cct gca gga acg acc	979
Ser Ile Asp Pro Asp Ala Thr Ala Ala Pro Val Pro Ala Gly Thr Thr	
280 285 290	
aaa gcc gaa gca gaa gca ccc gca gaa ttt tca aac gat tcc acc atc	1027
Lys Ala Glu Ala Glu Ala Pro Ala Glu Phe Ser Asn Asp Ser Thr Ile	
295 300 305	
atc cag gca cct ttg acc ggt gaa gct att gca ctg agc agc gtc agc	1075
Ile Gln Ala Pro Leu Thr Gly Glu Ala Ile Ala Leu Ser Ser Val Ser	
310 315 320 325	
gat gcc atg ttt gcc agc gga aag ctt ggc tgc ggc gtt gcc atc gtc	1123
Asp Ala Met Phe Ala Ser Gly Lys Leu Gly Ser Gly Val Ala Ile Val	
330 335 340	
cca acc aag ggg cag tta gtt tct ccg gtg agt gga aag att gtg gtg	1171
Pro Thr Lys Gly Gln Leu Val Ser Pro Val Ser Gly Lys Ile Val Val	
345 350 355	
gca ttc cca tct ggc cat gct ttc gca gtt cgc acc aag gct gag gat	1219
Ala Phe Pro Ser Gly His Ala Phe Ala Val Arg Thr Lys Ala Glu Asp	
360 365 370	
ggt tcc aat gtg gat atc ttg atg cac att ggt ttc gac aca gta aac	1267
Gly Ser Asn Val Asp Ile Leu Met His Ile Gly Phe Asp Thr Val Asn	
375 380 385	
ctc aac ggc acg cac ttt aac ccg ctg aag aag cag ggc gat gaa gtc	1315
Leu Asn Gly Thr His Phe Asn Pro Leu Lys Lys Gln Gly Asp Glu Val	
390 395 400 405	

27

aaa gca ggg gag ctg ctg tgt gaa ttc gat att gat gcc att aag gct 1363
 Lys Ala Gly Glu Leu Leu Cys Glu Phe Asp Ile Asp Ala Ile Lys Ala
 410 415 420

gca ggt tat gag gta acc acg ccg att gtt gtt tcg aat tac aag aaa 1411
 Ala Gly Tyr Glu Val Thr Thr Pro Ile Val Val Ser Asn Tyr Lys Lys
 425 430 435

acc gga cct gta aac act tac ggt ttg ggc gaa att gaa gcg gga gcc 1459
 Thr Gly Pro Val Asn Thr Tyr Gly Leu Gly Glu Ile Glu Ala Gly Ala
 440 445 450

aac ctg ctc aac gtc gca aag aaa gaa gcg gtg cca gca aca cca 1504
 Asn Leu Leu Asn Val Ala Lys Lys Glu Ala Val Pro Ala Thr Pro
 455 460 465

taagttgaaa ccttgagtgt tcg 1527

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<211> 468

<212> PRT

<213> Corynebacterium glutamicum

<400> 10

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Thr Met Ala Ala Gly Glu Met Pro Met Trp Ser Leu Phe Gly Leu Asp
 20 25 30

Val Ala Gln Ala Gly Tyr Gln Gly Thr Val Leu Pro Val Leu Val Val
 35 40 45

Ser Trp Ile Leu Ala Thr Ile Glu Lys Phe Leu His Lys Arg Leu Lys
 50 55 60

Gly Thr Ala Asp Phe Leu Ile Thr Pro Val Leu Thr Leu Leu Thr
 65 70 75 80

Gly Phe Leu Thr Phe Ile Ala Ile Gly Pro Ala Met Arg Trp Val Gly
 85 90 95

Asp Val Leu Ala His Gly Leu Gln Gly Leu Tyr Asp Phe Gly Gly Pro
 100 105 110

Val Gly Gly Leu Leu Phe Gly Leu Val Tyr Ser Pro Ile Val Ile Thr
 115 120 125

Gly Leu His Gln Ser Phe Pro Pro Ile Glu Leu Glu Leu Phe Asn Gln
 130 135 140

Gly Gly Ser Phe Ile Phe Ala Thr Ala Ser Met Ala Asn Ile Ala Gln
 145 150 155 160

28

Gly Ala Ala Cys Leu Ala Val Phe Phe Leu Ala Lys Ser Glu Lys Leu
 165 170 175
 Lys Gly Leu Ala Gly Ala Ser Gly Val Ser Ala Val Leu Gly Ile Thr
 180 185 190
 Glu Pro Ala Ile Phe Gly Val Asn Leu Arg Leu Arg Trp Pro Phe Phe
 195 200 205
 Ile Gly Ile Gly Thr Ala Ala Ile Gly Gly Ala Leu Ile Ala Leu Phe
 210 215 220
 Asn Ile Lys Ala Val Ala Leu Gly Ala Ala Gly Phe Leu Gly Val Val
 225 230 235 240
 Ser Ile Asp Ala Pro Asp Met Val Met Phe Leu Val Cys Ala Val Val
 245 250 255
 Thr Phe Phe Ile Ala Phe Gly Ala Ala Ile Ala Tyr Gly Leu Tyr Leu
 260 265 270
 Val Arg Arg Asn Gly Ser Ile Asp Pro Asp Ala Thr Ala Ala Pro Val
 275 280 285
 Pro Ala Gly Thr Thr Lys Ala Glu Ala Glu Ala Pro Ala Glu Phe Ser
 290 295 300
 Asn Asp Ser Thr Ile Ile Gln Ala Pro Leu Thr Gly Glu Ala Ile Ala
 305 310 315 320
 Leu Ser Ser Val Ser Asp Ala Met Phe Ala Ser Gly Lys Leu Gly Ser
 325 330 335
 Gly Val Ala Ile Val Pro Thr Lys Gly Gln Leu Val Ser Pro Val Ser
 340 345 350
 Gly Lys Ile Val Val Ala Phe Pro Ser Gly His Ala Phe Ala Val Arg
 355 360 365
 Thr Lys Ala Glu Asp Gly Ser Asn Val Asp Ile Leu Met His Ile Gly
 370 375 380
 Phe Asp Thr Val Asn Leu Asn Gly Thr His Phe Asn Pro Leu Lys Lys
 385 390 395 400
 Gln Gly Asp Glu Val Lys Ala Gly Glu Leu Leu Cys Glu Phe Asp Ile
 405 410 415
 Asp Ala Ile Lys Ala Ala Gly Tyr Glu Val Thr Thr Pro Ile Val Val
 420 425 430
 Ser Asn Tyr Lys Lys Thr Gly Pro Val Asn Thr Tyr Gly Leu Gly Glu
 435 440 445
 Ile Glu Ala Gly Ala Asn Leu Leu Asn Val Ala Lys Lys Glu Ala Val
 450 455 460

29

Pro Ala Thr Pro
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<210> 11

<211> 2187

<212> DNA

<213> Corynebacterium glutamicum

<220>

<221> CDS

<222> (101)..(2164)

<223> RXN01299

<400> 11

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gtgcaacggt cacccaagtc aaaggattga aagaatcagc atg aat agc gta aat 115
Met Asn Ser Val Asn
1 5

aat tcc tcg ctt gtc cgg ctg gat gtc gat ttc ggc gac tcc acc acg 163
Asn Ser Ser Leu Val Arg Leu Asp Val Asp Phe Gly Asp Ser Thr Thr
10 15 20

gat gtc atc aac aac ctt gcc act gtt att ttc gac gct ggc cga gct 211
Asp Val Ile Asn Asn Leu Ala Thr Val Ile Phe Asp Ala Gly Arg Ala
25 30 35

tcc tcc gcc gac gcc ctt gcc aaa gac gcg ctg gat cgt gaa gca aag 259
Ser Ser Ala Asp Ala Leu Ala Lys Asp Ala Leu Asp Arg Glu Ala Lys
40 45 50

tcc ggc acc ggc gtt cct ggt caa gtt gct atc ccc cac tgc cgt tcc 307
Ser Gly Thr Gly Val Pro Gly Gln Val Ala Ile Pro His Cys Arg Ser
55 60 65

gaa gcc gta tct gtc cct acc ttg ggc ttt gct cgc ctg agc aag ggt 355
Glu Ala Val Ser Val Pro Thr Leu Gly Phe Ala Arg Leu Ser Lys Gly
70 75 80 85

gtg gac ttc agc gga cct gat ggc gat gcc aac ttg gtg ttc ctc att 403
Val Asp Phe Ser Gly Pro Asp Gly Asp Ala Asn Leu Val Phe Leu Ile
90 95 100

gca gca cct gct ggc ggc ggc aaa gag cac ctg aag atc ctg tcc aag 451
Ala Ala Pro Ala Gly Gly Gly Lys Glu His Leu Lys Ile Leu Ser Lys
105 110 115

ctt gct cgc tcc ttg gtg aag aag gat ttc atc aag gct ctg cag gaa 499
Leu Ala Arg Ser Leu Val Lys Lys Asp Phe Ile Lys Ala Leu Gln Glu
120 125 130

30

gcc acc acc gag cag gaa atc gtc gac gtt gtc gat gcc gtg ctc aac	547
Ala Thr Thr Glu Gln Glu Ile Val Asp Val Val Asp Ala Val Leu Asn	
135 140 145	
cca gca cca aaa acc acc gag cca gct gca gct ccg gct gcg gcg gcg	595
Pro Ala Pro Lys Thr Thr Glu Pro Ala Ala Ala Pro Ala Ala Ala Ala	
150 155 160 165	
gtt gct gag agt ggg gcg gcg tcg aca agc gtt act cgt atc gtg gca	643
Val Ala Glu Ser Gly Ala Ala Ser Thr Ser Val Thr Arg Ile Val Ala	
170 175 180	
atc acc gca tgc cca acc ggt atc gca cac acc tac atg gct gcg gat	691
Ile Thr Ala Cys Pro Thr Gly Ile Ala His Thr Tyr Met Ala Ala Asp	
185 190 195	
tcc ctg acg caa aac gcg gaa ggc cgc gat gat gtg gaa ctc gtt gtg	739
Ser Leu Thr Gln Asn Ala Glu Gly Arg Asp Asp Val Glu Leu Val Val	
200 205 210	
gag act cag ggc tct tcc gct gtc acc cca gtc gat ccg aag atc atc	787
Glu Thr Gln Gly Ser Ser Ala Val Thr Pro Val Asp Pro Lys Ile Ile	
215 220 225	
gaa gct gcc gac gcc gtc atc ttc gcc acc gac gtg gga gtt aaa gac	835
Glu Ala Ala Asp Ala Val Ile Phe Ala Thr Asp Val Gly Val Lys Asp	
230 235 240 245	
cgc gag cgt ttc gct ggc aag cca gtc att gaa tcc ggc gtc aag cgc	883
Arg Glu Arg Phe Ala Gly Lys Pro Val Ile Glu Ser Gly Val Lys Arg	
250 255 260	
gcg atc aat gag cca gcc aag atg atc gac gag gcc atc gca gcc tcc	931
Ala Ile Asn Glu Pro Ala Lys Met Ile Asp Glu Ala Ile Ala Ala Ser	
265 270 275	
aag aac cca aac gcc cgc aag gtt tcc ggt tcc ggt gtc gcg gca tct	979
Lys Asn Pro Asn Ala Arg Lys Val Ser Gly Ser Gly Val Ala Ala Ser	
280 285 290	
gct gaa acc acc ggc gag aag ctc ggc tgg ggc aag cgc atc cag cag	1027
Ala Glu Thr Thr Gly Glu Lys Leu Gly Trp Gly Lys Arg Ile Gln Gln	
295 300 305	
gca gtc atg acc ggc gtg tcc tac atg gtt cca ttc gta gct gcc ggc	1075
Ala Val Met Thr Gly Val Ser Tyr Met Val Pro Phe Val Ala Ala Gly	
310 315 320 325	
ggc ctc ctg ttg gct ctc ggc ttc gca ttc ggt gga tac gac atg gcg	1123
Gly Leu Leu Leu Ala Leu Gly Phe Ala Phe Gly Gly Tyr Asp Met Ala	
330 335 340	

31

aac ggc tgg caa gca atc gcc acc cag ttc tct ctg acc aac ctg cca	1171
Asn Gly Trp Gln Ala Ile Ala Thr Gln Phe Ser Leu Thr Asn Leu Pro	
345 350 355	
ggc aac acc gtc gat gtt gac ggc gtg gcc atg acc ttc gag cgt tca	1219
Gly Asn Thr Val Asp Val Asp Gly Val Ala Met Thr Phe Glu Arg Ser	
360 365 370	
ggc ttc ctg ttg tac ttc ggc gca gtc ctg ttc gcc acc ggc caa gca	1267
Gly Phe Leu Leu Tyr Phe Gly Ala Val Leu Phe Ala Thr Gly Gln Ala	
375 380 385	
gcc atg ggc ttc atc gtg gca gcc ctg tct ggc tac acc gca tac gca	1315
Ala Met Gly Phe Ile Val Ala Ala Leu Ser Gly Tyr Thr Ala Tyr Ala	
390 395 400 405	
ctt gct gga cgc cca ggc atc gcg ccg ggc ttc gtc ggt ggc gcc atc	1363
Leu Ala Gly Arg Pro Gly Ile Ala Pro Gly Phe Val Gly Gly Ala Ile	
410 415 420	
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Ser Val Thr Ile Gly Ala Gly Phe Ile Gly Gly Leu Val Thr Gly Ile	
425 430 435	
ttg gct ggt ctc att gcc ctg tgg att ggc tcc tgg aag gtg cca cgc	1459
Leu Ala Gly Leu Ile Ala Leu Trp Ile Gly Ser Trp Lys Val Pro Arg	
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gtg gtg cag tca ctg atg cct gtg gtc atc atc ccg cta ctt acc tca	1507
Val Val Gln Ser Leu Met Pro Val Val Ile Ile Pro Leu Leu Thr Ser	
455 460 465	
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Val Val Val Gly Leu Val Met Tyr Leu Leu Leu Gly Arg Pro Leu Ala	
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Ser Ile Met Thr Gly Leu Gln Asp Trp Leu Ser Ser Met Ser Gly Ser	
490 495 500	
tcc gcc atc ttg ctg ggt atc atc ttg ggc ctc atg atg tgt ttc gac	1651
Ser Ala Ile Leu Leu Gly Ile Ile Leu Gly Leu Met Met Cys Phe Asp	
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ctc ggc gga cca gta aac aag gca gcc tac ctc ttt ggt acc gca ggc	1699
Leu Gly Gly Pro Val Asn Lys Ala Ala Tyr Leu Phe Gly Thr Ala Gly	
520 525 530	
ctg tct acc ggc gac caa gct tcc atg gaa atc atg gcc gcg atc atg	1747
Leu Ser Thr Gly Asp Gln Ala Ser Met Glu Ile Met Ala Ala Ile Met	
535 540 545	

32

gca gct ggc atg gtc cca cca atc gcg ttg tcc att gct acc ctg ctg 1795
 Ala Ala Gly Met Val Pro Pro Ile Ala Leu Ser Ile Ala Thr Leu Leu
 550 555 560 565

cgc aag aag ctg ttc acc cca gca gag caa gaa aac ggc aag tct tcc 1843
 Arg Lys Lys Leu Phe Thr Pro Ala Glu Gln Glu Asn Gly Lys Ser Ser
 570 575 580

tgg ctg ctt ggc ctg gca ttc gtc tcc gaa ggt gcc atc cca ttc gcc 1891
 Trp Leu Leu Gly Leu Ala Phe Val Ser Glu Gly Ala Ile Pro Phe Ala
 585 590 595

gca gct gac cca ttc cgt gtg atc cca gca atg atg gct ggc ggt gca 1939
 Ala Ala Asp Pro Phe Arg Val Ile Pro Ala Met Met Ala Gly Gly Ala
 600 605 610

acc act ggt gca atc tcc atg gca ctg ggc gtc ggc tct cgg gct cca 1987
 Thr Thr Gly Ala Ile Ser Met Ala Leu Gly Val Gly Ser Arg Ala Pro
 615 620 625

cac ggc ggt atc ttc gtg gtc tgg gca atc gaa cca tgg tgg ggc tgg 2035
 His Gly Gly Ile Phe Val Val Trp Ala Ile Glu Pro Trp Trp Gly Trp
 630 635 640 645

ctc atc gca ctt gca gca ggc acc atc gtg tcc acc atc gtt gtc atc 2083
 Leu Ile Ala Leu Ala Ala Gly Thr Ile Val Ser Thr Ile Val Val Ile
 650 655 660

gca ctg aag cag ttc tgg cca aac aag gcc gtc gct gca gaa gtc gcg 2131
 Ala Leu Lys Gln Phe Trp Pro Asn Lys Ala Val Ala Ala Glu Val Ala
 665 670 675

aag caa gaa gca caa caa gca gct gta aac gca taatcggacc ttgacccgat 2184
 Lys Gln Glu Ala Gln Gln Ala Ala Val Asn Ala
 680 685

gtc 2187

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 <212> PRT
 <213> Corynebacterium glutamicum

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 Gly Asp Ser Thr Thr Asp Val Ile Asn Asn Leu Ala Thr Val Ile Phe
 20 25 30
 Asp Ala Gly Arg Ala Ser Ser Ala Asp Ala Leu Ala Lys Asp Ala Leu
 35 40 45
 Asp Arg Glu Ala Lys Ser Gly Thr Gly Val Pro Gly Gln Val Ala Ile
 50 55 60

33

Pro	His	Cys	Arg	Ser	Glu	Ala	Val	Ser	Val	Pro	Thr	Leu	Gly	Phe	Ala	65	70	75	80
Arg	Leu	Ser	Lys	Gly	Val	Asp	Phe	Ser	Gly	Pro	Asp	Gly	Asp	Ala	Asn	85	90	95	
Leu	Val	Phe	Leu	Ile	Ala	Ala	Pro	Ala	Gly	Gly	Gly	Lys	Glu	His	Leu	100	105	110	
Lys	Ile	Leu	Ser	Lys	Leu	Ala	Arg	Ser	Leu	Val	Lys	Lys	Asp	Phe	Ile	115	120	125	
Lys	Ala	Leu	Gln	Glu	Ala	Thr	Thr	Glu	Gln	Glu	Ile	Val	Asp	Val	Val	130	135	140	
Asp	Ala	Val	Leu	Asn	Pro	Ala	Pro	Lys	Thr	Thr	Glu	Pro	Ala	Ala	Ala	145	150	155	160
Pro	Ala	Ala	Ala	Ala	Val	Ala	Glu	Ser	Gly	Ala	Ala	Ser	Thr	Ser	Val	165	170		175
Thr	Arg	Ile	Val	Ala	Ile	Thr	Ala	Cys	Pro	Thr	Gly	Ile	Ala	His	Thr	180	185		190
Tyr	Met	Ala	Ala	Asp	Ser	Leu	Thr	Gln	Asn	Ala	Glu	Gly	Arg	Asp	Asp	195	200	205	
Val	Glu	Leu	Val	Val	Glu	Thr	Gln	Gly	Ser	Ser	Ala	Val	Thr	Pro	Val	210	215	220	
Asp	Pro	Lys	Ile	Ile	Glu	Ala	Ala	Asp	Ala	Val	Ile	Phe	Ala	Thr	Asp	225	230	235	240
Val	Gly	Val	Lys	Asp	Arg	Glu	Arg	Phe	Ala	Gly	Lys	Pro	Val	Ile	Glu	245	250		255
Ser	Gly	Val	Lys	Arg	Ala	Ile	Asn	Glu	Pro	Ala	Lys	Met	Ile	Asp	Glu	260	265		270
Ala	Ile	Ala	Ala	Ser	Lys	Asn	Pro	Asn	Ala	Arg	Lys	Val	Ser	Gly	Ser	275	280		285
Gly	Val	Ala	Ala	Ser	Ala	Glu	Thr	Thr	Gly	Glu	Lys	Leu	Gly	Trp	Gly	290	295	300	
Lys	Arg	Ile	Gln	Gln	Ala	Val	Met	Thr	Gly	Val	Ser	Tyr	Met	Val	Pro	305	310	315	320
Phe	Val	Ala	Ala	Gly	Gly	Leu	Leu	Leu	Ala	Leu	Gly	Phe	Ala	Phe	Gly	325	330		335
Gly	Tyr	Asp	Met	Ala	Asn	Gly	Trp	Gln	Ala	Ile	Ala	Thr	Gln	Phe	Ser	340	345		350
Leu	Thr	Asn	Leu	Pro	Gly	Asn	Thr	Val	Asp	Val	Asp	Gly	Val	Ala	Met	355	360		365

34

Thr	Phe	Glu	Arg	Ser	Gly	Phe	Leu	Leu	Tyr	Phe	Gly	Ala	Val	Leu	Phe	370	375	380	
Ala	Thr	Gly	Gln	Ala	Ala	Met	Gly	Phe	Ile	Val	Ala	Ala	Leu	Ser	Gly	385	390	395	400
Tyr	Thr	Ala	Tyr	Ala	Leu	Ala	Gly	Arg	Pro	Gly	Ile	Ala	Pro	Gly	Phe	405	410	415	
Val	Gly	Gly	Ala	Ile	Ser	Val	Thr	Ile	Gly	Ala	Gly	Phe	Ile	Gly	Gly	420	425	430	
Leu	Val	Thr	Gly	Ile	Leu	Ala	Gly	Leu	Ile	Ala	Leu	Trp	Ile	Gly	Ser	435	440	445	
Trp	Lys	Val	Pro	Arg	Val	Val	Gln	Ser	Leu	Met	Pro	Val	Val	Ile	Ile	450	455	460	
Pro	Leu	Leu	Thr	Ser	Val	Val	Val	Gly	Leu	Val	Met	Tyr	Leu	Leu	Leu	465	470	475	480
Gly	Arg	Pro	Leu	Ala	Ser	Ile	Met	Thr	Gly	Leu	Gln	Asp	Trp	Leu	Ser	485	490	495	
Ser	Met	Ser	Gly	Ser	Ser	Ala	Ile	Leu	Leu	Gly	Ile	Ile	Leu	Gly	Leu	500	505	510	
Met	Met	Cys	Phe	Asp	Leu	Gly	Gly	Pro	Val	Asn	Lys	Ala	Ala	Tyr	Leu	515	520	525	
Phe	Gly	Thr	Ala	Gly	Leu	Ser	Thr	Gly	Asp	Gln	Ala	Ser	Met	Glu	Ile	530	535	540	
Met	Ala	Ala	Ile	Met	Ala	Ala	Gly	Met	Val	Pro	Pro	Ile	Ala	Leu	Ser	545	550	555	560
Ile	Ala	Thr	Leu	Leu	Arg	Lys	Lys	Leu	Phe	Thr	Pro	Ala	Glu	Gln	Glu	565	570	575	
Asn	Gly	Lys	Ser	Ser	Trp	Leu	Leu	Gly	Leu	Ala	Phe	Val	Ser	Glu	Gly	580	585	590	
Ala	Ile	Pro	Phe	Ala	Ala	Ala	Asp	Pro	Phe	Arg	Val	Ile	Pro	Ala	Met	595	600	605	
Met	Ala	Gly	Gly	Ala	Thr	Thr	Gly	Ala	Ile	Ser	Met	Ala	Leu	Gly	Val	610	615	620	
Gly	Ser	Arg	Ala	Pro	His	Gly	Gly	Ile	Phe	Val	Val	Trp	Ala	Ile	Glu	625	630	635	640
Pro	Trp	Trp	Gly	Trp	Leu	Ile	Ala	Leu	Ala	Ala	Gly	Thr	Ile	Val	Ser	645	650	655	
Thr	Ile	Val	Val	Ile	Ala	Leu	Lys	Gln	Phe	Trp	Pro	Asn	Lys	Ala	Val	660	665	670	

35

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<212> DNA

<213> *Corynebacterium glutamicum*

<220>

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<222> (1)..(393)

<223> RXA00951

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 Ile Gln Ala Ile Leu Glu Lys Ala Ala Ala Pro Ala Lys Gln Lys Ala
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cct gct gtg gct cct gct gta aca ccc act gac gct cct gca gcc tca 96
 Pro Ala Val Ala Pro Ala Val Thr Pro Thr Asp Ala Pro Ala Ala Ser
 20 25 30

gtc caa tcc aaa acc cac gac aag atc ctc acc gtc tgt ggc aac ggc 144
 Val Gln Ser Lys Thr His Asp Lys Ile Leu Thr Val Cys Gly Asn Gly
 35 40 45

ttg ggt acc tcc ctc ttc ctc aaa aac acc ctt gag caa gtt ttc gac 192
 Leu Gly Thr Ser Leu Phe Leu Lys Asn Thr Leu Glu Gln Val Phe Asp
 50 55 60

acc tgg ggt tgg ggt cca tac atg acg gtg gag gca acc gac act atc 240
 Thr Trp Gly Trp Gly Pro Tyr Met Thr Val Glu Ala Thr Asp Thr Ile
 65 70 75 80

tcc gcc aag ggc aaa gcc aag gaa gct gat ctc atc atg acc tct ggt 288
 Ser Ala Lys Gly Lys Ala Lys Glu Ala Asp Leu Ile Met Thr Ser Gly
 85 90 95

gaa atc gcc cgc acg ttg ggt gat gtt gga atc ccg gtt cac gtg atc 336
 Glu Ile Ala Arg Thr Leu Gly Asp Val Gly Ile Pro Val His Val Ile
 100 105 110

aat gac ttc acg agc acc gat gaa atc gat gct gcg ctt cgt gaa cgc 384
 Asn Asp Phe Thr Ser Thr Asp Glu Ile Asp Ala Ala Leu Arg Glu Arg
 115 120 125

tac gac atc taactacttt aaaaggacga aaa 416
 Tyr Asp Ile
 130

<210> 14

<211> 131

<212> PRT

<213> *Corynebacterium glutamicum*

36

<400> 14

Ile Gln Ala Ile Leu Glu Lys Ala Ala Ala Pro Ala Lys Gln Lys Ala
 1 5 10 15

Pro Ala Val Ala Pro Ala Val Thr Pro Thr Asp Ala Pro Ala Ala Ser
 20 25 30

Val Gln Ser Lys Thr His Asp Lys Ile Leu Thr Val Cys Gly Asn Gly
 35 40 45

Leu Gly Thr Ser Leu Phe Leu Lys Asn Thr Leu Glu Gln Val Phe Asp
 50 55 60

Thr Trp Gly Trp Gly Pro Tyr Met Thr Val Glu Ala Thr Asp Thr Ile
 65 70 75 80

Ser Ala Lys Gly Lys Ala Lys Glu Ala Asp Leu Ile Met Thr Ser Gly
 85 90 95

Glu Ile Ala Arg Thr Leu Gly Asp Val Gly Ile Pro Val His Val Ile
 100 105 110

Asn Asp Phe Thr Ser Thr Asp Glu Ile Asp Ala Ala Leu Arg Glu Arg
 115 120 125

Tyr Asp Ile
 130

<210> 15

<211> 1827

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<213> Corynebacterium glutamicum

<220>

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<222> (101)..(1804)

<223> RXN01244

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 Val Ala Thr Val Ala
 1 5

gat gtg aat caa gac act gta ctg aag ggc acc ggc gtt gtc ggt gga 163
 Asp Val Asn Gln Asp Thr Val Leu Lys Gly Thr Gly Val Val Gly Gly
 10 15 20

gtc cgt tat gca agc gcg gtg tgg att acc cca cgc ccc gaa cta ccc 211
 Val Arg Tyr Ala Ser Ala Val Trp Ile Thr Pro Arg Pro Glu Leu Pro
 25 30 35

37

caa gca ggc gaa gtc gtc gcc gaa gaa aac cgt gaa gca gag cag gag	259
Gln Ala Gly Glu Val Val Ala Glu Glu Asn Arg Glu Ala Glu Gln Glu	
40 45 50	
cgt ttc gac gcc gct gca gcc aca gtc tct tct cgt ttg ctt gag cgc	307
Arg Phe Asp Ala Ala Ala Ala Thr Val Ser Ser Arg Leu Leu Glu Arg	
55 60 65	
tcc gaa gct gct gaa gga cca gca gct gag gtg ctt aaa gct act gct	355
Ser Glu Ala Ala Glu Gly Pro Ala Ala Glu Val Leu Lys Ala Thr Ala	
70 75 80 85	
ggc atg gtc aat gac cgt ggc tgg cgt aag gct gtc atc aag ggt gtc	403
Gly Met Val Asn Asp Arg Gly Trp Arg Lys Ala Val Ile Lys Gly Val	
90 95 100	
aag ggt ggt cac cct gcg gaa tac gcc gtg gtt gca gca aca acc aag	451
Lys Gly Gly His Pro Ala Glu Tyr Ala Val Val Ala Ala Thr Thr Lys	
105 110 115	
ttc atc tcc atg ttc gaa gcc gca ggc ggc ctg atc gcg gag cgc acc	499
Phe Ile Ser Met Phe Glu Ala Ala Gly Gly Leu Ile Ala Glu Arg Thr	
120 125 130	
aca gac ttg cgc gac atc cgc gac cgc gtc atc gca gaa ctt cgt ggc	547
Thr Asp Leu Arg Asp Ile Arg Asp Arg Val Ile Ala Glu Leu Arg Gly	
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gat gaa gag cca ggt ctg cca gct gtt tcc gga cag gtc att ctc ttt	595
Asp Glu Glu Pro Gly Leu Pro Ala Val Ser Gly Gln Val Ile Leu Phe	
150 155 160 165	
gca gat gac ctc tcc cca gca gac acc gcg gca cta gac aca gat ctc	643
Ala Asp Asp Leu Ser Pro Ala Asp Thr Ala Ala Leu Asp Thr Asp Leu	
170 175 180	
ttt gtg gga ctt gtc act gag ctg ggt ggc cca acg agc cac acc gcg	691
Phe Val Gly Leu Val Thr Glu Leu Gly Gly Pro Thr Ser His Thr Ala	
185 190 195	
atc atc gca cgc cag ctc aac gtg cct tgc atc gtc gca tcc ggc gcc	739
Ile Ile Ala Arg Gln Leu Asn Val Pro Cys Ile Val Ala Ser Gly Ala	
200 205 210	
ggc atc aag gac atc aag tcc ggc gaa aag gtg ctt atc gac ggc agc	787
Gly Ile Lys Asp Ile Lys Ser Gly Glu Lys Val Leu Ile Asp Gly Ser	
215 220 225	
ctc ggc acc att gac cgc aac gcg gac gaa gct gaa gca acc aag ctc	835
Leu Gly Thr Ile Asp Arg Asn Ala Asp Glu Ala Glu Ala Thr Lys Leu	
230 235 240 245	

38

gtc tcc gag tcc ctc gag cgc gct gct cgc atc gcc gag tgg aag ggt	883
Val Ser Glu Ser Leu Glu Arg Ala Ala Arg Ile Ala Glu Trp Lys Gly	
250 255 260	
cct gca caa acc aag gac ggc tac cgc gtt cag ctg ttg gcc aac gtc	931
Pro Ala Gln Thr Lys Asp Gly Tyr Arg Val Gln Leu Leu Ala Asn Val	
265 270 275	
caa gac ggc aac tct gca cag cag gct gca cag acc gaa gca gaa ggc	979
Gln Asp Gly Asn Ser Ala Gln Gln Ala Ala Gln Thr Glu Ala Glu Gly	
280 285 290	
atc ggc ctg ttc cgc acc gaa ctg tgc ttc ctt tcc gcc acc gaa gag	1027
Ile Gly Leu Phe Arg Thr Glu Leu Cys Phe Leu Ser Ala Thr Glu Glu	
295 300 305	
cca agc gtt gat gag cag gct gcg gtc tac tca aag gtg ctt gaa gca	1075
Pro Ser Val Asp Glu Gln Ala Ala Val Tyr Ser Lys Val Leu Glu Ala	
310 315 320 325	
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Phe Pro Glu Ser Lys Val Val Val Arg Ser Leu Asp Ala Gly Ser Asp	
330 335 340	
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Lys Pro Val Pro Phe Ala Ser Met Ala Asp Glu Met Asn Pro Ala Leu	
345 350 355	
ggt gtt cgt ggc ctg cgt atc gca cgt gga cag gtt gat ctg ctg act	1219
Gly Val Arg Gly Leu Arg Ile Ala Arg Gly Gln Val Asp Leu Leu Thr	
360 365 370	
cgc cag ctc gac gca att gcg aag gcc agc gaa gaa ctc ggc cgt ggc	1267
Arg Gln Leu Asp Ala Ile Ala Lys Ala Ser Glu Glu Leu Gly Arg Gly	
375 380 385	
gac gac gcc cca acc tgg gtt atg gct cca atg gtg gct acc gct tat	1315
Asp Asp Ala Pro Thr Trp Val Met Ala Pro Met Val Ala Thr Ala Tyr	
390 395 400 405	
gaa gca aag tgg ttt gct gac atg tgc cgt gag cgt ggc cta atc gcc	1363
Glu Ala Lys Trp Phe Ala Asp Met Cys Arg Glu Arg Gly Leu Ile Ala	
410 415 420	
ggc gcc atg atc gaa gtt cca gca gca tcc ctg atg gca gac aag atc	1411
Gly Ala Met Ile Glu Val Pro Ala Ala Ser Leu Met Ala Asp Lys Ile	
425 430 435	
atg cct cac ctg gac ttt gtt tcc atc ggt acc aac gac ctg acc cag	1459
Met Pro His Leu Asp Phe Val Ser Ile Gly Thr Asn Asp Leu Thr Gln	
440 445 450	

39

tac acc atg gca gcg gac cgc atg tct cct gag ctt gcc tac ctg acc 1507
 Tyr Thr Met Ala Ala Asp Arg Met Ser Pro Glu Leu Ala Tyr Leu Thr
 455 460 465

 gat cct tgg cag cca gca gtc ctg cgc ctg atc aag cac acc tgt gac 1555
 Asp Pro Trp Gln Pro Ala Val Leu Arg Leu Ile Lys His Thr Cys Asp
 470 475 480 485

 gaa ggt gct cgc ttt aac acc ccg gtc ggt gtt tgt ggt gaa gca gca 1603
 Glu Gly Ala Arg Phe Asn Thr Pro Val Gly Val Cys Gly Glu Ala Ala
 490 495 500

 gca gac cca ctg ttg gca act gtc ctc acc ggt ctt ggc gtg aac tcc 1651
 Ala Asp Pro Leu Leu Ala Thr Val Leu Thr Gly Leu Gly Val Asn Ser
 505 510 515

 ctg tcc gca gca tcc act gct ctc gca gca gtc ggt gca aag ctg tca 1699
 Leu Ser Ala Ala Ser Thr Ala Leu Ala Ala Val Gly Ala Lys Leu Ser
 520 525 530

 gag gtc acc ctg gaa acc tgt aag aag gca gca gaa gca gca ctt gac 1747
 Glu Val Thr Leu Glu Thr Cys Lys Lys Ala Ala Glu Ala Ala Leu Asp
 535 540 545

 gct gaa ggt gca act gaa gca cgc gat gct gta cgc gca gtg atc gac 1795
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 550 555 560 565

 gca gca gtc taaaccactg ttgagctaaa aag 1827
 Ala Ala Val

<210> 16

<211> 568

<212> PRT

<213> *Corynebacterium glutamicum*

<400> 16

Val Ala Thr Val Ala Asp Val Asn Gln Asp Thr Val Leu Lys Gly Thr
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 20 25 30

 Arg Pro Glu Leu Pro Gln Ala Gly Glu Val Val Ala Glu Glu Asn Arg
 35 40 45

 Glu Ala Glu Gln Glu Arg Phe Asp Ala Ala Ala Thr Val Ser Ser
 50 55 60

 Arg Leu Leu Glu Arg Ser Glu Ala Ala Glu Gly Pro Ala Ala Glu Val
 65 70 75 80

 Leu Lys Ala Thr Ala Gly Met Val Asn Asp Arg Gly Trp Arg Lys Ala
 85 90 95

40

Val	Ile	Lys	Gly	Val	Lys	Gly	Gly	His	Pro	Ala	Glu	Tyr	Ala	Val	Val	100	105	110	
Ala	Ala	Thr	Thr	Lys	Phe	Ile	Ser	Met	Phe	Glu	Ala	Ala	Gly	Gly	Leu	115	120	125	
Ile	Ala	Glu	Arg	Thr	Thr	Asp	Leu	Arg	Asp	Ile	Arg	Asp	Arg	Val	Ile	130	135	140	
Ala	Glu	Leu	Arg	Gly	Asp	Glu	Glu	Pro	Gly	Leu	Pro	Ala	Val	Ser	Gly	145	150	155	160
Gln	Val	Ile	Leu	Phe	Ala	Asp	Asp	Leu	Ser	Pro	Ala	Asp	Thr	Ala	Ala	165	170	175	
Leu	Asp	Thr	Asp	Leu	Phe	Val	Gly	Leu	Val	Thr	Glu	Leu	Gly	Gly	Pro	180	185	190	
Thr	Ser	His	Thr	Ala	Ile	Ile	Ala	Arg	Gln	Leu	Asn	Val	Pro	Cys	Ile	195	200	205	
Val	Ala	Ser	Gly	Ala	Gly	Ile	Lys	Asp	Ile	Lys	Ser	Gly	Glu	Lys	Val	210	215	220	
Leu	Ile	Asp	Gly	Ser	Leu	Gly	Thr	Ile	Asp	Arg	Asn	Ala	Asp	Glu	Ala	225	230	235	240
Glu	Ala	Thr	Lys	Leu	Val	Ser	Glu	Ser	Leu	Glu	Arg	Ala	Ala	Arg	Ile	245	250	255	
Ala	Glu	Trp	Lys	Gly	Pro	Ala	Gln	Thr	Lys	Asp	Gly	Tyr	Arg	Val	Gln	260	265	270	
Leu	Leu	Ala	Asn	Val	Gln	Asp	Gly	Asn	Ser	Ala	Gln	Gln	Ala	Ala	Gln	275	280	285	
Thr	Glu	Ala	Glu	Gly	Ile	Gly	Leu	Phe	Arg	Thr	Glu	Leu	Cys	Phe	Leu	290	295	300	
Ser	Ala	Thr	Glu	Glu	Pro	Ser	Val	Asp	Glu	Gln	Ala	Ala	Val	Tyr	Ser	305	310	315	320
Lys	Val	Leu	Glu	Ala	Phe	Pro	Glu	Ser	Lys	Val	Val	Val	Arg	Ser	Leu	325	330	335	
Asp	Ala	Gly	Ser	Asp	Lys	Pro	Val	Pro	Phe	Ala	Ser	Met	Ala	Asp	Glu	340	345	350	
Met	Asn	Pro	Ala	Leu	Gly	Val	Arg	Gly	Leu	Arg	Ile	Ala	Arg	Gly	Gln	355	360	365	
Val	Asp	Leu	Leu	Thr	Arg	Gln	Leu	Asp	Ala	Ile	Ala	Lys	Ala	Ser	Glu	370	375	380	
Glu	Leu	Gly	Arg	Gly	Asp	Asp	Ala	Pro	Thr	Trp	Val	Met	Ala	Pro	Met	385	390	395	400

41

Val Ala Thr Ala Tyr Glu Ala Lys Trp Phe Ala Asp Met Cys Arg Glu
 405 410 415

Arg Gly Leu Ile Ala Gly Ala Met Ile Glu Val Pro Ala Ala Ser Leu
 420 425 430

Met Ala Asp Lys Ile Met Pro His Leu Asp Phe Val Ser Ile Gly Thr
 435 440 445

Asn Asp Leu Thr Gln Tyr Thr Met Ala Ala Asp Arg Met Ser Pro Glu
 450 455 460

Leu Ala Tyr Leu Thr Asp Pro Trp Gln Pro Ala Val Leu Arg Leu Ile
 465 470 475 480

Lys His Thr Cys Asp Glu Gly Ala Arg Phe Asn Thr Pro Val Gly Val
 485 490 495

Cys Gly Glu Ala Ala Ala Asp Pro Leu Leu Ala Thr Val Leu Thr Gly
 500 505 510

Leu Gly Val Asn Ser Leu Ser Ala Ala Ser Thr Ala Leu Ala Ala Val
 515 520 525

Gly Ala Lys Leu Ser Glu Val Thr Leu Glu Thr Cys Lys Lys Ala Ala
 530 535 540

Glu Ala Ala Leu Asp Ala Glu Gly Ala Thr Glu Ala Arg Asp Ala Val
 545 550 555 560

Arg Ala Val Ile Asp Ala Ala Val
 565

<210> 17

<211> 390

<212> DNA

<213> *Corynebacterium glutamicum*

<220>

<221> CDS

<222> (101)..(367)

<223> RXA01300

<400> 17

gatcgacatt aaatcccctc ccttggggggg tttaactaac aaatcgctgc gccctaattcc 60

gttcggatta acggcgtagc aacacgaaag gacactttcc atg gct tcc aag act 115
 Met Ala Ser Lys Thr
 1 5

gta acc gtc ggt tcc tcc gtt ggc ctg cac gca cgt cca gca tcc atc 163
 Val Thr Val Gly Ser Ser Val Gly Leu His Ala Arg Pro Ala Ser Ile
 10 15 20

42

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atc gct gaa gcg gct gct gag tac gac gac gaa atc ttg ctg acc ctg 211
Ile Ala Glu Ala Ala Ala Glu Tyr Asp Asp Glu Ile Leu Leu Thr Leu
      25              30              35

ggt ggc tcc gat gat gac gaa gag acc gac gcg tcc tct tcc ctc atg 259
Val Gly Ser Asp Asp Asp Glu Glu Thr Asp Ala Ser Ser Ser Leu Met
      40              45              50

atc atg gcg ctg ggc gca gag cac ggc aac gaa gtt acc gtc acc tcc 307
Ile Met Ala Leu Gly Ala Glu His Gly Asn Glu Val Thr Val Thr Ser
      55              60              65

gac aac gct gaa gct gtt gag aag atc gct gcg ctt atc gca cag gac 355
Asp Asn Ala Glu Ala Val Glu Lys Ile Ala Ala Leu Ile Ala Gln Asp
      70              75              80              85

ctt gac gct gag taaacaacgc tctgcttggt aaa 390
Leu Asp Ala Glu

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<210> 18

<211> 89

<212> PRT

<213> *Corynebacterium glutamicum*

<400> 18

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Met Ala Ser Lys Thr Val Thr Val Gly Ser Ser Val Gly Leu His Ala
  1              5              10              15

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Arg Pro Ala Ser Ile Ile Ala Glu Ala Ala Glu Tyr Asp Asp Glu
      20              25              30

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```

Ile Leu Leu Thr Leu Val Gly Ser Asp Asp Asp Glu Glu Thr Asp Ala
      35              40              45

```

```

Ser Ser Ser Leu Met Ile Met Ala Leu Gly Ala Glu His Gly Asn Glu
      50              55              60

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```

Val Thr Val Thr Ser Asp Asn Ala Glu Ala Val Glu Lys Ile Ala Ala
      65              70              75              80

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Leu Ile Ala Gln Asp Leu Asp Ala Glu
      85

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<210> 19

<211> 508

<212> DNA

<213> *Corynebacterium glutamicum*

<220>

<221> CDS

<222> (101)..(508)

<223> RXN03002

43

<400> 19

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accctatccg aatcaacatg cagtgaatta acatctactt atg ttt gta ctc aaa 115
 Met Phe Val Leu Lys
 1 5

gat ctg cta aag gca gaa cgc ata gaa ctc gac cgc acg gtc acc gat 163
 Asp Leu Leu Lys Ala Glu Arg Ile Glu Leu Asp Arg Thr Val Thr Asp
 10 15 20

tgg cgt gaa ggc atc cgc gcc gca ggt gta ctc cta gaa aag aca aac 211
 Trp Arg Glu Gly Ile Arg Ala Ala Gly Val Leu Leu Glu Lys Thr Asn
 25 30 35

agc att gat tcc gcc tac acc gat gcc atg atc gcc agc gtg gaa gaa 259
 Ser Ile Asp Ser Ala Tyr Thr Asp Ala Met Ile Ala Ser Val Glu Glu
 40 45 50

aaa ggc ccc tac att gtg gtc gct cca ggt ttc gct ttc gcg cac gcc 307
 Lys Gly Pro Tyr Ile Val Val Ala Pro Gly Phe Ala Phe Ala His Ala
 55 60 65

cgc ccc agc aga gca gtc cgc gag acc gct atg tcg tgg gtg cgc ctg 355
 Arg Pro Ser Arg Ala Val Arg Glu Thr Ala Met Ser Trp Val Arg Leu
 70 75 80 85

gcc tcc cct gtt tcc ttc ggt cac agt aag aat gat ccc ctc aat ctc 403
 Ala Ser Pro Val Ser Phe Gly His Ser Lys Asn Asp Pro Leu Asn Leu
 90 95 100

atc gtt gct ctc gct gcc aaa gat gcc acc gca cat acc caa gcg atg 451
 Ile Val Ala Leu Ala Ala Lys Asp Ala Thr Ala His Thr Gln Ala Met
 105 110 115

gcg gca ttg gct aaa gct tta gga aaa tac cga aag gat ctc gac gag 499
 Ala Ala Leu Ala Lys Ala Leu Gly Lys Tyr Arg Lys Asp Leu Asp Glu
 120 125 130

gca caa agt 508
 Ala Gln Ser
 135

<210> 20

<211> 136

<212> PRT

<213> *Corynebacterium glutamicum*

<400> 20

Met Phe Val Leu Lys Asp Leu Leu Lys Ala Glu Arg Ile Glu Leu Asp
 1 5 10 15

Arg Thr Val Thr Asp Trp Arg Glu Gly Ile Arg Ala Ala Gly Val Leu
 20 25 30

44

Leu Glu Lys Thr Asn Ser Ile Asp Ser Ala Tyr Thr Asp Ala Met Ile
35 40 45

Ala Ser Val Glu Glu Lys Gly Pro Tyr Ile Val Val Ala Pro Gly Phe
50 55 60

Ala Phe Ala His Ala Arg Pro Ser Arg Ala Val Arg Glu Thr Ala Met
65 70 75 80

Ser Trp Val Arg Leu Ala Ser Pro Val Ser Phe Gly His Ser Lys Asn
85 90 95

Asp Pro Leu Asn Leu Ile Val Ala Leu Ala Ala Lys Asp Ala Thr Ala
100 105 110

His Thr Gln Ala Met Ala Ala Leu Ala Lys Ala Leu Gly Lys Tyr Arg
115 120 125

Lys Asp Leu Asp Glu Ala Gln Ser
130 135

<210> 21

<211> 789

<212> DNA

<213> *Corynebacterium glutamicum*

<220>

<221> CDS

<222> (14) .. (766)

<223> RXC00953

<400> 21

cttgcattcc ccaatg gcg cca cca acg gta ggc aac tac atc atg cag tcc 52

Met Ala Pro Pro Thr Val Gly Asn Tyr Ile Met Gln Ser

1 **5** **10**

ttc act caa ggt ctg cag ttc ggc gtt gca gtt gcc gtg att ctc ttt 100
Phe Thr Gln Gly Leu Gln Phe Gly Val Ala Val Ala Val Ile Leu Phe
15 20 25

ggt gtc cgc acc att ctt ggt gaa ctg gtc ccc gca ttc caa ggt att 148
Gly Val Arg Thr Ile Leu Gly Glu Leu Val Pro Ala Phe Gln Gly Ile
30 35 40 45

gct gcg aag gtt gtt ccc gga gct atc ccc gca ttg gat gca ccg atc 196
Ala Ala Lys Val Val Pro Gly Ala Ile Pro Ala Leu Asp Ala Pro Ile
50 55 60

gtg ttc ccc tac gcg cag aac gcc gtt ctc att ggt ttc ttg tct tcc 244
Val Phe Pro Tyr Ala Gln Asn Ala Val Leu Ile Gly Phe Leu Ser Ser
65 70 75

ttc gtc ggt ggc ttg gtt ggc ctg act gtt ctt gca tgc tgg ctg aac 292
Phe Val Gly Gly Leu Val Gly Leu Thr Val Leu Ala Ser Trp Leu Asn
80 85 90

45

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cca gct ttt ggt gtc gcg ttg att ctg cct ggt ttg gtc ccc cac ttc 340
Pro Ala Phe Gly Val Ala Leu Ile Leu Pro Gly Leu Val Pro His Phe
    95                100                105

ttc act ggt ggc gcg gcg ggc gtt tac ggt aat gcc acg ggt ggt cgt 388
Phe Thr Gly Gly Ala Ala Gly Val Tyr Gly Asn Ala Thr Gly Gly Arg
110                115                120                125

cga gga gca gta ttt ggc gcc ttt gcc aac ggt ctt ctg att acc ttc 436
Arg Gly Ala Val Phe Gly Ala Phe Ala Asn Gly Leu Leu Ile Thr Phe
                130                135                140

ctc cct gct ttc ctg ctt ggt gtg ctt ggt tcc ttc ggg tca gag aac 484
Leu Pro Ala Phe Leu Leu Gly Val Leu Gly Ser Phe Gly Ser Glu Asn
                145                150                155

acc act ttc ggt gat gcg gac ttt ggt tgg ttc gga atc gtt gtt ggt 532
Thr Thr Phe Gly Asp Ala Asp Phe Gly Trp Phe Gly Ile Val Val Gly
    160                165                170

tct gca gcc aag gtg gaa ggt gct ggc ggg ctc atc ttg ttg ctc atc 580
Ser Ala Ala Lys Val Glu Gly Ala Gly Gly Leu Ile Leu Leu Leu Ile
    175                180                185

atc gca gcg gtt ctt ctg ggt ggc gcg atg gtc ttc cag aag cgc gtc 628
Ile Ala Ala Val Leu Leu Gly Gly Ala Met Val Phe Gln Lys Arg Val
190                195                200                205

gtg aat ggg cac tgg gat cca gct ccc aac cgt gag cgc gtg gag aag 676
Val Asn Gly His Trp Asp Pro Ala Pro Asn Arg Glu Arg Val Glu Lys
                210                215                220

gcg gaa gct gat gcc act cca acg gct ggg gct cgg acc tac cct aag 724
Ala Glu Ala Asp Ala Thr Pro Thr Ala Gly Ala Arg Thr Tyr Pro Lys
                225                230                235

att gct cct ccg gcg ggc gct cct acc cca ccg gct cga agc 766
Ile Ala Pro Pro Ala Gly Ala Pro Thr Pro Pro Ala Arg Ser
    240                245                250

taagatctcc aaaaccctga gat 789

<210> 22
<211> 251
<212> PRT
<213> Corynebacterium glutamicum

<400> 22
Met Ala Pro Pro Thr Val Gly Asn Tyr Ile Met Gln Ser Phe Thr Gln
    1                5                10                15

Gly Leu Gln Phe Gly Val Ala Val Ala Val Ile Leu Phe Gly Val Arg
    20                25                30

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46

Thr Ile Leu Gly Glu Leu Val Pro Ala Phe Gln Gly Ile Ala Ala Lys
 35 40 45
 Val Val Pro Gly Ala Ile Pro Ala Leu Asp Ala Pro Ile Val Phe Pro
 50 55 60
 Tyr Ala Gln Asn Ala Val Leu Ile Gly Phe Leu Ser Ser Phe Val Gly
 65 70 75 80
 Gly Leu Val Gly Leu Thr Val Leu Ala Ser Trp Leu Asn Pro Ala Phe
 85 90 95
 Gly Val Ala Leu Ile Leu Pro Gly Leu Val Pro His Phe Phe Thr Gly
 100 105 110
 Gly Ala Ala Gly Val Tyr Gly Asn Ala Thr Gly Gly Arg Arg Gly Ala
 115 120 125
 Val Phe Gly Ala Phe Ala Asn Gly Leu Leu Ile Thr Phe Leu Pro Ala
 130 135 140
 Phe Leu Leu Gly Val Leu Gly Ser Phe Gly Ser Glu Asn Thr Thr Phe
 145 150 155 160
 Gly Asp Ala Asp Phe Gly Trp Phe Gly Ile Val Val Gly Ser Ala Ala
 165 170 175
 Lys Val Glu Gly Ala Gly Gly Leu Ile Leu Leu Leu Ile Ile Ala Ala
 180 185 190
 Val Leu Leu Gly Gly Ala Met Val Phe Gln Lys Arg Val Val Asn Gly
 195 200 205
 His Trp Asp Pro Ala Pro Asn Arg Glu Arg Val Glu Lys Ala Glu Ala
 210 215 220
 Asp Ala Thr Pro Thr Ala Gly Ala Arg Thr Tyr Pro Lys Ile Ala Pro
 225 230 235 240
 Pro Ala Gly Ala Pro Thr Pro Pro Ala Arg Ser
 245 250

<210> 23

<211> 553

<212> DNA

<213> *Corynebacterium glutamicum*

<220>

<221> CDS

<222> (101)..(553)

<223> RXC03001

<400> 23

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47

acgctacgac atctaactac tttaaaagga cgaaaatatt atg gac tgg tta acc	115
Met Asp Trp Leu Thr	
1 5	
att cct ctt ttc ctc gtt aat gaa atc ctt gcg gtt ccg gct ttc ctc	163
Ile Pro Leu Phe Leu Val Asn Glu Ile Leu Ala Val Pro Ala Phe Leu	
10 15 20	
atc ggt atc atc acc gcc gtg gga ttg ggt gcc atg ggg cgt tcc gtc	211
Ile Gly Ile Ile Thr Ala Val Gly Leu Gly Ala Met Gly Arg Ser Val	
25 30 35	
ggt cag gtt atc ggt gga gca atc aaa gca acg ttg ggc ttt ttg ctc	259
Gly Gln Val Ile Gly Gly Ala Ile Lys Ala Thr Leu Gly Phe Leu Leu	
40 45 50	
att ggt gcg ggt gcc acg ttg gtc act gcc tcc ctg gag cca ctg ggt	307
Ile Gly Ala Gly Ala Thr Leu Val Thr Ala Ser Leu Glu Pro Leu Gly	
55 60 65	
gcg atg atc atg ggt gcc aca ggc atg cgt ggt gtt gtc cca acg aat	355
Ala Met Ile Met Gly Ala Thr Gly Met Arg Gly Val Val Pro Thr Asn	
70 75 80 85	
gaa gcc atc gcc gga atc gca cag gct gaa tac ggc gcg cag gtg gcg	403
Glu Ala Ile Ala Gly Ile Ala Gln Ala Glu Tyr Gly Ala Gln Val Ala	
90 95 100	
tgg ctg atg att ctg ggc ttc gcc atc tct ttg gtg ttg gct cgt ttc	451
Trp Leu Met Ile Leu Gly Phe Ala Ile Ser Leu Val Leu Ala Arg Phe	
105 110 115	
acc aac ctg cgt tat gtc ttg ctc aac gga cac cac gtg ctg ttg atg	499
Thr Asn Leu Arg Tyr Val Leu Leu Asn Gly His His Val Leu Leu Met	
120 125 130	
tgc acc atg ctc acc atg gtc ttg gcc acc gga aga gtt gat gcg tgg	547
Cys Thr Met Leu Thr Met Val Leu Ala Thr Gly Arg Val Asp Ala Trp	
135 140 145	
atc ttc	553
Ile Phe	
150	
<210> 24	
<211> 151	
<212> PRT	
<213> Corynebacterium glutamicum	
<400> 24	
Met Asp Trp Leu Thr Ile Pro Leu Phe Leu Val Asn Glu Ile Leu Ala	
1 5 10 15	

48

Val Pro Ala Phe Leu Ile Gly Ile Ile Thr Ala Val Gly Leu Gly Ala
20 25 30

Met Gly Arg Ser Val Gly Gln Val Ile Gly Gly Ala Ile Lys Ala Thr
35 40 45

Leu Gly Phe Leu Leu Ile Gly Ala Gly Ala Thr Leu Val Thr Ala Ser
50 55 60

Leu Glu Pro Leu Gly Ala Met Ile Met Gly Ala Thr Gly Met Arg Gly
65 70 75 80

Val Val Pro Thr Asn Glu Ala Ile Ala Gly Ile Ala Gln Ala Glu Tyr
85 90 95

Gly Ala Gln Val Ala Trp Leu Met Ile Leu Gly Phe Ala Ile Ser Leu
100 105 110

Val Leu Ala Arg Phe Thr Asn Leu Arg Tyr Val Leu Leu Asn Gly His
115 120 125

His Val Leu Leu Met Cys Thr Met Leu Thr Met Val Leu Ala Thr Gly
130 135 140

Arg Val Asp Ala Trp Ile Phe
145 150

<210> 25

<211> 2172

<212> DNA

<213> Corynebacterium glutamicum

<220>

<221> CDS

<222> (101)..(2149)

<223> RXN01943

<400> 25

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tgcccgcggg agacagaccc tacgtttaga aaggtttgac atg gcg tcc aaa ctg 115
Met Ala Ser Lys Leu
1 5

acg acg aca tcg caa cat att ctg gaa aac ctt ggt gga cca gac aat 163
Thr Thr Thr Ser Gln His Ile Leu Glu Asn Leu Gly Gly Pro Asp Asn
10 15 20

att act tcg atg act cac tgt gcg act cgc ctt cgc ttc caa gtg aag 211
Ile Thr Ser Met Thr His Cys Ala Thr Arg Leu Arg Phe Gln Val Lys
25 30 35

gat caa tcc att gtt gat caa caa gaa att gac tcc gac cca tca gtt 259
Asp Gln Ser Ile Val Asp Gln Gln Glu Ile Asp Ser Asp Pro Ser Val
40 45 50

49

ctt ggc gta gta ccc caa gga tcc acc ggt atg cag gtg gtg atg ggt	307
Leu Gly Val Val Pro Gln Gly Ser Thr Gly Met Gln Val Val Met Gly	
55 60 65	
gga tct gtt gca aac tat tac caa gaa atc ctc aaa ctt gat gga atg	355
Gly Ser Val Ala Asn Tyr Tyr Gln Glu Ile Leu Lys Leu Asp Gly Met	
70 75 80 85	
aag cac ttc gcc gac ggt gaa gct aca gag agt tca tcc aag aag gaa	403
Lys His Phe Ala Asp Gly Glu Ala Thr Glu Ser Ser Ser Lys Lys Glu	
90 95 100	
tac ggc gga gtc cgt ggc aag tac tcg tgg att gac tac gcc ttc gag	451
Tyr Gly Gly Val Arg Gly Lys Tyr Ser Trp Ile Asp Tyr Ala Phe Glu	
105 110 115	
ttc ttg tct gat act ttc cga cca atc ctg tgg gcc ctg ctt ggt gcc	499
Phe Leu Ser Asp Thr Phe Arg Pro Ile Leu Trp Ala Leu Leu Gly Ala	
120 125 130	
tca ctg att att acc ttg ttg gtt ctt gcg gat act ttc ggt ttg caa	547
Ser Leu Ile Ile Thr Leu Leu Val Leu Ala Asp Thr Phe Gly Leu Gln	
135 140 145	
gac ttc cgc gct cca atg gat gag cag cct gat act tat gta ttc ctg	595
Asp Phe Arg Ala Pro Met Asp Glu Gln Pro Asp Thr Tyr Val Phe Leu	
150 155 160 165	
cac tcc atg tgg cgc tcg gtc ttc tac ttc ctg cca att atg gtt ggt	643
His Ser Met Trp Arg Ser Val Phe Tyr Phe Leu Pro Ile Met Val Gly	
170 175 180	
gcc acc gca gct cga aag ctc ggc gca aac gag tgg att ggt gca gct	691
Ala Thr Ala Ala Arg Lys Leu Gly Ala Asn Glu Trp Ile Gly Ala Ala	
185 190 195	
att cca gcc gca ctt ctt act cca gaa ttc ttg gca ctg ggt tct gcc	739
Ile Pro Ala Ala Leu Leu Thr Pro Glu Phe Leu Ala Leu Gly Ser Ala	
200 205 210	
ggc gat acc gtc aca gtc ttt ggc ctg cca atg gtt ctg aat gac tac	787
Gly Asp Thr Val Thr Val Phe Gly Leu Pro Met Val Leu Asn Asp Tyr	
215 220 225	
tcc gga cag gta ttc cca ccg ctg att gca gca att ggt ctg tac tgg	835
Ser Gly Gln Val Phe Pro Pro Leu Ile Ala Ala Ile Gly Leu Tyr Trp	
230 235 240 245	
gtg gaa aag gga ctg aag aag atc atc cct gaa gca gtc caa atg gtg	883
Val Glu Lys Gly Leu Lys Lys Ile Ile Pro Glu Ala Val Gln Met Val	
250 255 260	

50

ttc gtc cca ttc ttc tcc ctg ctg att atg atc cca gcg acc gca ttc	931
Phe Val Pro Phe Phe Ser Leu Leu Ile Met Ile Pro Ala Thr Ala Phe	
265 270 275	
ctg ctt gga cct ttc ggc atc ggt gtt ggt aac gga att tcc aac ctg	979
Leu Leu Gly Pro Phe Gly Ile Gly Val Gly Asn Gly Ile Ser Asn Leu	
280 285 290	
ctt gaa gcg att aac aac ttc agc cca ttt att ctt tcc atc gtt atc	1027
Leu Glu Ala Ile Asn Asn Phe Ser Pro Phe Ile Leu Ser Ile Val Ile	
295 300 305	
cca ttg ctc tac cca ttc ttg gtt cca ctt gga ttg cac tgg cca cta	1075
Pro Leu Leu Tyr Pro Phe Leu Val Pro Leu Gly Leu His Trp Pro Leu	
310 315 320 325	
aac gcc atc atg atc cag aac atc aac acc ctg ggt tac gac ttc att	1123
Asn Ala Ile Met Ile Gln Asn Ile Asn Thr Leu Gly Tyr Asp Phe Ile	
330 335 340	
cag gga cca atg ggt gcc tgg aac ttc gcc tgc ttc ggc ctg gtc acc	1171
Gln Gly Pro Met Gly Ala Trp Asn Phe Ala Cys Phe Gly Leu Val Thr	
345 350 355	
ggc gtg ttc ttg ctc tcc att aag gaa cga aac aag gcc atg cgt cag	1219
Gly Val Phe Leu Leu Ser Ile Lys Glu Arg Asn Lys Ala Met Arg Gln	
360 365 370	
gtt tcc ctg ggt ggc atg ttg gct ggt ttg ctc ggc ggc att tcc gag	1267
Val Ser Leu Gly Gly Met Leu Ala Gly Leu Leu Gly Gly Ile Ser Glu	
375 380 385	
cct tcc ctc tac ggt gtt ctg ctc cga ttc aag aag acc tac ttc cgc	1315
Pro Ser Leu Tyr Gly Val Leu Leu Arg Phe Lys Lys Thr Tyr Phe Arg	
390 395 400 405	
ctc ctg ccg ggt tgt ttg gca ggc ggt atc gtg atg ggc atc ttc gac	1363
Leu Leu Pro Gly Cys Leu Ala Gly Gly Ile Val Met Gly Ile Phe Asp	
410 415 420	
atc aag gcg tac gct ttc gtg ttc acc tcc ttg ctt acc atc cca gca	1411
Ile Lys Ala Tyr Ala Phe Val Phe Thr Ser Leu Leu Thr Ile Pro Ala	
425 430 435	
atg gac cca tgg ttg ggc tac acc att ggt atc gca gtt gca ttc ttc	1459
Met Asp Pro Trp Leu Gly Tyr Thr Ile Gly Ile Ala Val Ala Phe Phe	
440 445 450	
gtt tcc atg ttc ctt gtt ctc gca ctg gac tac cgt tcc aac gaa gag	1507
Val Ser Met Phe Leu Val Leu Ala Leu Asp Tyr Arg Ser Asn Glu Glu	
455 460 465	

51

cgc gat gag gca cgt gca aag gtt gct gct gac aag cag gca gaa gaa	1555
Arg Asp Glu Ala Arg Ala Lys Val Ala Ala Asp Lys Gln Ala Glu Glu	
470 475 480 485	
gat ctg aag gca gaa gct aat gca act cct gca gct cca gta gct gct	1603
Asp Leu Lys Ala Glu Ala Asn Ala Thr Pro Ala Ala Pro Val Ala Ala	
490 495 500	
gca ggt gcg gga gcc ggt gca ggt gca gga gcc gct gct ggc gct gca	1651
Ala Gly Ala Gly Ala Gly Ala Gly Ala Gly Ala Ala Ala Gly Ala Ala	
505 510 515	
acc gcc gtg gca gct aag ccg aag ctg gcc gct ggg gaa gta gtg gac	1699
Thr Ala Val Ala Ala Lys Pro Lys Leu Ala Ala Gly Glu Val Val Asp	
520 525 530	
att gtt tcc cca ctc gaa ggc aag gca att cca ctt tct gaa gta cct	1747
Ile Val Ser Pro Leu Glu Gly Lys Ala Ile Pro Leu Ser Glu Val Pro	
535 540 545	
gac cca atc ttt gca gca ggc aag ctt gga cca ggc att gca atc caa	1795
Asp Pro Ile Phe Ala Ala Gly Lys Leu Gly Pro Gly Ile Ala Ile Gln	
550 555 560 565	
cca act gga aac acc gtt gtt gct cca gca gac gct act gtc atc ctt	1843
Pro Thr Gly Asn Thr Val Val Ala Pro Ala Asp Ala Thr Val Ile Leu	
570 575 580	
gtc cag aaa tct gga cac gca gtg gca ttg cgc tta gat agc gga gtt	1891
Val Gln Lys Ser Gly His Ala Val Ala Leu Arg Leu Asp Ser Gly Val	
585 590 595	
gaa atc ctt gtc cac gtt gga ttg gac acc gtg caa ttg ggc ggc gaa	1939
Glu Ile Leu Val His Val Gly Leu Asp Thr Val Gln Leu Gly Gly Glu	
600 605 610	
ggc ttc acc gtt cac gtt gag cgc agg cag caa gtc aag gcg ggg gat	1987
Gly Phe Thr Val His Val Glu Arg Arg Gln Gln Val Lys Ala Gly Asp	
615 620 625	
cca ctg atc act ttt gac gct gac ttc att cga tcc aag gat cta cct	2035
Pro Leu Ile Thr Phe Asp Ala Asp Phe Ile Arg Ser Lys Asp Leu Pro	
630 635 640 645	
ttg atc acc cca gtt gtg gtg tct aac gcc gcg aaa ttc ggt gaa att	2083
Leu Ile Thr Pro Val Val Val Ser Asn Ala Ala Lys Phe Gly Glu Ile	
650 655 660	
gaa ggt att cct gca gat cag gca aat tct tcc acg act gtg atc aag	2131
Glu Gly Ile Pro Ala Asp Gln Ala Asn Ser Ser Thr Thr Val Ile Lys	
665 670 675	

52

gtc aac ggc aag aac gag taacctggga tccatgttgc gca

2172

Val Asn Gly Lys Asn Glu

680

<210> 26

<211> 683

<212> PRT

<213> *Corynebacterium glutamicum*

<400> 26

Met Ala Ser Lys Leu Thr Thr Thr Ser Gln His Ile Leu Glu Asn Leu

1

5

10

15

Gly Gly Pro Asp Asn Ile Thr Ser Met Thr His Cys Ala Thr Arg Leu

20

25

30

Arg Phe Gln Val Lys Asp Gln Ser Ile Val Asp Gln Gln Glu Ile Asp

35

40

45

Ser Asp Pro Ser Val Leu Gly Val Val Pro Gln Gly Ser Thr Gly Met

50

55

60

Gln Val Val Met Gly Gly Ser Val Ala Asn Tyr Tyr Gln Glu Ile Leu

65

70

75

80

Lys Leu Asp Gly Met Lys His Phe Ala Asp Gly Glu Ala Thr Glu Ser

85

90

95

Ser Ser Lys Lys Glu Tyr Gly Gly Val Arg Gly Lys Tyr Ser Trp Ile

100

105

110

Asp Tyr Ala Phe Glu Phe Leu Ser Asp Thr Phe Arg Pro Ile Leu Trp

115

120

125

Ala Leu Leu Gly Ala Ser Leu Ile Ile Thr Leu Leu Val Leu Ala Asp

130

135

140

Thr Phe Gly Leu Gln Asp Phe Arg Ala Pro Met Asp Glu Gln Pro Asp

145

150

155

160

Thr Tyr Val Phe Leu His Ser Met Trp Arg Ser Val Phe Tyr Phe Leu

165

170

175

Pro Ile Met Val Gly Ala Thr Ala Ala Arg Lys Leu Gly Ala Asn Glu

180

185

190

Trp Ile Gly Ala Ala Ile Pro Ala Ala Leu Leu Thr Pro Glu Phe Leu

195

200

205

Ala Leu Gly Ser Ala Gly Asp Thr Val Thr Val Phe Gly Leu Pro Met

210

215

220

Val Leu Asn Asp Tyr Ser Gly Gln Val Phe Pro Pro Leu Ile Ala Ala

225

230

235

240

53

Ile Gly Leu Tyr Trp Val Glu Lys Gly Leu Lys Lys Ile Ile Pro Glu	245	250	255
Ala Val Gln Met Val Phe Val Pro Phe Phe Ser Leu Leu Ile Met Ile	260	265	270
Pro Ala Thr Ala Phe Leu Leu Gly Pro Phe Gly Ile Gly Val Gly Asn	275	280	285
Gly Ile Ser Asn Leu Leu Glu Ala Ile Asn Asn Phe Ser Pro Phe Ile	290	295	300
Leu Ser Ile Val Ile Pro Leu Leu Tyr Pro Phe Leu Val Pro Leu Gly	305	310	315
Leu His Trp Pro Leu Asn Ala Ile Met Ile Gln Asn Ile Asn Thr Leu	325	330	335
Gly Tyr Asp Phe Ile Gln Gly Pro Met Gly Ala Trp Asn Phe Ala Cys	340	345	350
Phe Gly Leu Val Thr Gly Val Phe Leu Leu Ser Ile Lys Glu Arg Asn	355	360	365
Lys Ala Met Arg Gln Val Ser Leu Gly Gly Met Leu Ala Gly Leu Leu	370	375	380
Gly Gly Ile Ser Glu Pro Ser Leu Tyr Gly Val Leu Leu Arg Phe Lys	385	390	395
Lys Thr Tyr Phe Arg Leu Leu Pro Gly Cys Leu Ala Gly Gly Ile Val	405	410	415
Met Gly Ile Phe Asp Ile Lys Ala Tyr Ala Phe Val Phe Thr Ser Leu	420	425	430
Leu Thr Ile Pro Ala Met Asp Pro Trp Leu Gly Tyr Thr Ile Gly Ile	435	440	445
Ala Val Ala Phe Phe Val Ser Met Phe Leu Val Leu Ala Leu Asp Tyr	450	455	460
Arg Ser Asn Glu Glu Arg Asp Glu Ala Arg Ala Lys Val Ala Ala Asp	465	470	475
Lys Gln Ala Glu Glu Asp Leu Lys Ala Glu Ala Asn Ala Thr Pro Ala	485	490	495
Ala Pro Val Ala Ala Ala Gly Ala Gly Ala Gly Ala Gly Ala Gly Ala	500	505	510
Ala Ala Gly Ala Ala Thr Ala Val Ala Ala Lys Pro Lys Leu Ala Ala	515	520	525
Gly Glu Val Val Asp Ile Val Ser Pro Leu Glu Gly Lys Ala Ile Pro	530	535	540

54

Leu Ser Glu Val Pro Asp Pro Ile Phe Ala Ala Gly Lys Leu Gly Pro
 545 550 555 560
 Gly Ile Ala Ile Gln Pro Thr Gly Asn Thr Val Val Ala Pro Ala Asp
 565 570 575
 Ala Thr Val Ile Leu Val Gln Lys Ser Gly His Ala Val Ala Leu Arg
 580 585 590
 Leu Asp Ser Gly Val Glu Ile Leu Val His Val Gly Leu Asp Thr Val
 595 600 605
 Gln Leu Gly Gly Glu Gly Phe Thr Val His Val Glu Arg Arg Gln Gln
 610 615 620
 Val Lys Ala Gly Asp Pro Leu Ile Thr Phe Asp Ala Asp Phe Ile Arg
 625 630 635 640
 Ser Lys Asp Leu Pro Leu Ile Thr Pro Val Val Val Ser Asn Ala Ala
 645 650 655
 Lys Phe Gly Glu Ile Glu Gly Ile Pro Ala Asp Gln Ala Asn Ser Ser
 660 665 670
 Thr Thr Val Ile Lys Val Asn Gly Lys Asn Glu
 675 680

<210> 27

<211> 372

<212> DNA

<213> Corynebacterium glutamicum

<220>

<221> CDS

<222> (101)..(349)

<223> RXA01503

<400> 27

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 cgacctatca aattctttat gctgcaggcg atgccttttc atg ttc ttg gca gtc 115
 Met Phe Leu Ala Val
 1 5
 att ttg gcg att act gcg gct cgt aaa ttc ggt gcc aat gtc ttt aca 163
 Ile Leu Ala Ile Thr Ala Ala Arg Lys Phe Gly Ala Asn Val Phe Thr
 10 15 20
 tca gtc gca ctc gct ggt gca ttg ctg cac aca cag ctt cag gca gta 211
 Ser Val Ala Leu Ala Gly Ala Leu Leu His Thr Gln Leu Gln Ala Val
 25 30 35
 acc gtg ttg gtt gac ggt gaa ctc cag tcg atg act ctg gtg gct ttc 259
 Thr Val Leu Val Asp Gly Glu Leu Gln Ser Met Thr Leu Val Ala Phe
 40 45 50

caa aag gct ggt aat gac gtc acc ttc ctg ggc att cca gtg gtg ctg 307
Gln Lys Ala Gly Asn Asp Val Thr Phe Leu Gly Ile Pro Val Val Leu
55 60 65

cag ttg gcg ttg cat gta gcg agt ttg atg aag ttg tcg cga 349
Gln Leu Ala Leu His Val Ala Ser Leu Met Lys Leu Ser Arg
70 75 80

taagaggagg ggcgtgtcgg tct 372

<210> 28

<211> 83

<212> PRT

<213> *Corynebacterium glutamicum*

<400> 28

Met Phe Leu Ala Val Ile Leu Ala Ile Thr Ala Ala Arg Lys Phe Gly
1 5 10 15

Ala Asn Val Phe Thr Ser Val Ala Leu Ala Gly Ala Leu Leu His Thr
20 25 30

Gln Leu Gln Ala Val Thr Val Leu Val Asp Gly Glu Leu Gln Ser Met
35 40 45

Thr Leu Val Ala Phe Gln Lys Ala Gly Asn Asp Val Thr Phe Leu Gly
50 55 60

Ile Pro Val Val Leu Gln Leu Ala Leu His Val Ala Ser Leu Met Lys
65 70 75 80

Leu Ser Arg

<210> 29

<211> 1578

<212> DNA

<213> *Corynebacterium glutamicum*

<220>

<221> CDS

<222> (101) .. (1555)

<223> RXN00351

<400> 29

gaaggctgct gctaagaaaa cgaccaagaa gaccactaag aaaactacta aaaagaccac 60

cgcaaagaag accacaaaga agtcttaagc cggatcttat atg gat gat tcc aat 115
Met Asp Asp Ser Asn
1 5

agc ttt gta gtt gtt gct aac cgt ctg cca gtg gat atg act gtc cac 163
 Ser Phe Val Val Val Ala Asn Arg Leu Pro Val Asp Met Thr Val His
 10 15 20

56

cca gat ggt agc tat agc atc tcc ccc agc ccc ggt ggc ctt gtc acg	211
Pro Asp Gly Ser Tyr Ser Ile Ser Pro Ser Pro Gly Gly Leu Val Thr	
25 30 35	
ggg ctt tcc ccc gtt ctg gaa caa cat cgt gga tgt tgg gtc gga tgg	259
Gly Leu Ser Pro Val Leu Glu Gln His Arg Gly Cys Trp Val Gly Trp	
40 45 50	
cct gga act gta gat gtt gca ccc gaa cca ttt cga aca gat acg ggt	307
Pro Gly Thr Val Asp Val Ala Pro Glu Pro Phe Arg Thr Asp Thr Gly	
55 60 65	
gtt ttg ctg cac cct gtt gtc ctc act gca agt gac tat gaa ggc ttc	355
Val Leu Leu His Pro Val Val Leu Thr Ala Ser Asp Tyr Glu Gly Phe	
70 75 80 85	
tac gag ggc ttt tca aac gca acg ctg tgg cct ctt ttc cac gat ctg	403
Tyr Glu Gly Phe Ser Asn Ala Thr Leu Trp Pro Leu Phe His Asp Leu	
90 95 100	
att gtt act ccg gtg tac aac acc gat tgg tgg cat gcg ttt cgg gag	451
Ile Val Thr Pro Val Tyr Asn Thr Asp Trp Trp His Ala Phe Arg Glu	
105 110 115	
gta aac ctc aag ttc gct gaa gcc gtg agc caa gtg gcg gca cac ggt	499
Val Asn Leu Lys Phe Ala Glu Ala Val Ser Gln Val Ala Ala His Gly	
120 125 130	
gcc act gtg tgg gtg cag gac tat cag ctg ttg ctg gtt cct ggc att	547
Ala Thr Val Trp Val Gln Asp Tyr Gln Leu Leu Val Pro Gly Ile	
135 140 145	
ttg cgc cag atg cgc cct gat ttg aag atc ggt ttc ttc ctc cac att	595
Leu Arg Gln Met Arg Pro Asp Leu Lys Ile Gly Phe Phe Leu His Ile	
150 155 160 165	
ccc ttc cct tcc cct gat ctg ttc cgt cag ctg ccg tgg cgt gaa gag	643
Pro Phe Pro Ser Pro Asp Leu Phe Arg Gln Leu Pro Trp Arg Glu Glu	
170 175 180	
att gtt cga ggc atg ctg ggc gca gat ttg gtg gga ttc cat ttg gtt	691
Ile Val Arg Gly Met Leu Gly Ala Asp Leu Val Gly Phe His Leu Val	
185 190 195	
caa aac gca gaa aac ttc ctt gcg tta acc cag cag gtt gcc ggc act	739
Gln Asn Ala Glu Asn Phe Leu Ala Leu Thr Gln Gln Val Ala Gly Thr	
200 205 210	
gcc ggg tct cat gtg ggt cag ccg gac acc ttg cag gtc agt ggt gaa	787
Ala Gly Ser His Val Gly Gln Pro Asp Thr Leu Gln Val Ser Gly Glu	
215 220 225	

57

gca ttg gtg cgt gag att ggc gct cat gtt gaa acc gct gac gga agg	835
Ala Leu Val Arg Glu Ile Gly Ala His Val Glu Thr Ala Asp Gly Arg	
230 235 240 245	
cga gtt agc gtc ggg gcg ttc ccg atc tcg att gat gtt gaa atg ttt	883
Arg Val Ser Val Gly Ala Phe Pro Ile Ser Ile Asp Val Glu Met Phe	
250 255 260	
ggg gag gcg tcg aaa agc gcc gtt ctt gat ctt tta aaa acg ctc gac	931
Gly Glu Ala Ser Lys Ser Ala Val Leu Asp Leu Leu Lys Thr Leu Asp	
265 270 275	
gag ccg gaa acc gta ttc ctg ggc gtt gac cga ctg gac tac acc aag	979
Glu Pro Glu Thr Val Phe Leu Gly Val Asp Arg Leu Asp Tyr Thr Lys	
280 285 290	
ggc att ttg cag cgc ctg ctt gcg ttt gag gaa ctg ctg gaa tcc ggc	1027
Gly Ile Leu Gln Arg Leu Leu Ala Phe Glu Glu Leu Leu Glu Ser Gly	
295 300 305	
gcg ttg gag gcc gac aaa gct gtg ttg ctg cag gtc gcg acg cct tcg	1075
Ala Leu Glu Ala Asp Lys Ala Val Leu Leu Gln Val Ala Thr Pro Ser	
310 315 320 325	
cgt gag cgc att gat cac tat cgt gtg tcg cgt tcg cag gtc gag gaa	1123
Arg Glu Arg Ile Asp His Tyr Arg Val Ser Arg Ser Gln Val Glu Glu	
330 335 340	
gcc gtc ggc cgt atc aat ggt cgt ttc ggt cgc atg ggg cgt ccc gtg	1171
Ala Val Gly Arg Ile Asn Gly Arg Phe Gly Arg Met Gly Arg Pro Val	
345 350 355	
gtg cat tat cta cac agg tca ttg agc aaa aat gat ctc cag gtg ctg	1219
Val His Tyr Leu His Arg Ser Leu Ser Lys Asn Asp Leu Gln Val Leu	
360 365 370	
tat acc gca gcc gat gtc atg ctg gtt acg cct ttt aaa gac ggt atg	1267
Tyr Thr Ala Ala Asp Val Met Leu Val Thr Pro Phe Lys Asp Gly Met	
375 380 385	
aac ttg gtg gct aaa gaa ttc gtg gcc aac cac cgc gac ggc act ggt	1315
Asn Leu Val Ala Lys Glu Phe Val Ala Asn His Arg Asp Gly Thr Gly	
390 395 400 405	
gct ttg gtg ctg tcc gaa ttt gcc ggc gcg gcc act gag ctg acc ggt	1363
Ala Leu Val Leu Ser Glu Phe Ala Gly Ala Ala Thr Glu Leu Thr Gly	
410 415 420	
gcg tat tta tgc aac cca ttt gat gtg gaa tcc atc aaa cgg caa atg	1411
Ala Tyr Leu Cys Asn Pro Phe Asp Val Glu Ser Ile Lys Arg Gln Met	
425 430 435	

58

gtg gca gct gtc cat gat ttg aag cac aat ccg gaa tct gcg gca acg 1459
 Val Ala Ala Val His Asp Leu Lys His Asn Pro Glu Ser Ala Ala Thr
 440 445 450

cga atg aaa acg aac agc gag cag gtc tat acc cac gac gtc aac gtg 1507
 Arg Met Lys Thr Asn Ser Glu Gln Val Tyr Thr His Asp Val Asn Val
 455 460 465

tgg gct aat agt ttc ctg gat tgt ttg gca cag tcg gga gaa aac tca 1555
 Trp Ala Asn Ser Phe Leu Asp Cys Leu Ala Gln Ser Gly Glu Asn Ser
 470 475 480 485

tgaaccgcgc acgaatcgcg acc 1578

<210> 30

<211> 485

<212> PRT

<213> Corynebacterium glutamicum

<400> 30

Met Asp Asp Ser Asn Ser Phe Val Val Val Ala Asn Arg Leu Pro Val
 1 5 10 15

Asp Met Thr Val His Pro Asp Gly Ser Tyr Ser Ile Ser Pro Ser Pro
 20 25 30

Gly Gly Leu Val Thr Gly Leu Ser Pro Val Leu Glu Gln His Arg Gly
 35 40 45

Cys Trp Val Gly Trp Pro Gly Thr Val Asp Val Ala Pro Glu Pro Phe
 50 55 60

Arg Thr Asp Thr Gly Val Leu Leu His Pro Val Val Leu Thr Ala Ser
 65 70 75 80

Asp Tyr Glu Gly Phe Tyr Glu Gly Phe Ser Asn Ala Thr Leu Trp Pro
 85 90 95

Leu Phe His Asp Leu Ile Val Thr Pro Val Tyr Asn Thr Asp Trp Trp
 100 105 110

His Ala Phe Arg Glu Val Asn Leu Lys Phe Ala Glu Ala Val Ser Gln
 115 120 125

Val Ala Ala His Gly Ala Thr Val Trp Val Gln Asp Tyr Gln Leu Leu
 130 135 140

Leu Val Pro Gly Ile Leu Arg Gln Met Arg Pro Asp Leu Lys Ile Gly
 145 150 155 160

Phe Phe Leu His Ile Pro Phe Pro Ser Pro Asp Leu Phe Arg Gln Leu
 165 170 175

Pro Trp Arg Glu Glu Ile Val Arg Gly Met Leu Gly Ala Asp Leu Val
 180 185 190

59

Gly Phe His Leu Val Gln Asn Ala Glu Asn Phe Leu Ala Leu Thr Gln
 195 200 205
 Gln Val Ala Gly Thr Ala Gly Ser His Val Gly Gln Pro Asp Thr Leu
 210 215 220
 Gln Val Ser Gly Glu Ala Leu Val Arg Glu Ile Gly Ala His Val Glu
 225 230 235 240
 Thr Ala Asp Gly Arg Arg Val Ser Val Gly Ala Phe Pro Ile Ser Ile
 245 250 255
 Asp Val Glu Met Phe Gly Glu Ala Ser Lys Ser Ala Val Leu Asp Leu
 260 265 270
 Leu Lys Thr Leu Asp Glu Pro Glu Thr Val Phe Leu Gly Val Asp Arg
 275 280 285
 Leu Asp Tyr Thr Lys Gly Ile Leu Gln Arg Leu Leu Ala Phe Glu Glu
 290 295 300
 Leu Leu Glu Ser Gly Ala Leu Glu Ala Asp Lys Ala Val Leu Leu Gln
 305 310 315 320
 Val Ala Thr Pro Ser Arg Glu Arg Ile Asp His Tyr Arg Val Ser Arg
 325 330 335
 Ser Gln Val Glu Glu Ala Val Gly Arg Ile Asn Gly Arg Phe Gly Arg
 340 345 350
 Met Gly Arg Pro Val Val His Tyr Leu His Arg Ser Leu Ser Lys Asn
 355 360 365
 Asp Leu Gln Val Leu Tyr Thr Ala Ala Asp Val Met Leu Val Thr Pro
 370 375 380
 Phe Lys Asp Gly Met Asn Leu Val Ala Lys Glu Phe Val Ala Asn His
 385 390 395 400
 Arg Asp Gly Thr Gly Ala Leu Val Leu Ser Glu Phe Ala Gly Ala Ala
 405 410 415
 Thr Glu Leu Thr Gly Ala Tyr Leu Cys Asn Pro Phe Asp Val Glu Ser
 420 425 430
 Ile Lys Arg Gln Met Val Ala Ala Val His Asp Leu Lys His Asn Pro
 435 440 445
 Glu Ser Ala Ala Thr Arg Met Lys Thr Asn Ser Glu Gln Val Tyr Thr
 450 455 460
 His Asp Val Asn Val Trp Ala Asn Ser Phe Leu Asp Cys Leu Ala Gln
 465 470 475 480
 Ser Gly Glu Asn Ser
 485

60

<210> 31

<211> 891

<212> DNA

<213> Corynebacterium glutamicum

<220>

<221> CDS

<222> (101)..(868)

<223> RXA00347

<400> 31

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aaagtgccag	gggttctgtg	ggatccgtac	actggttccc	atg	act	ttg	act	att	115
				Met	Thr	Leu	Thr	Ile	
				1				5	

gag	gaa	atc	gcc	aag	acc	aaa	aag	ctt	ttg	gtt	gtg	tcc	gat	ttt	gat	163
Glu	Glu	Ile	Ala	Lys	Thr	Lys	Lys	Leu	Leu	Val	Val	Ser	Asp	Phe	Asp	
				10				15						20		

gga	acc	atc	gca	gga	ttt	agc	aag	gac	gct	tac	aac	gtt	cct	atc	aac	211
Gly	Thr	Ile	Ala	Gly	Phe	Ser	Lys	Asp	Ala	Tyr	Asn	Val	Pro	Ile	Asn	
				25				30					35			

cag	aaa	tcc	ctc	aag	gcg	gta	aaa	gac	ctc	tcc	caa	caa	gca	gac	act	259
Gln	Lys	Ser	Leu	Lys	Ala	Val	Lys	Asp	Leu	Ser	Gln	Gln	Ala	Asp	Thr	
				40				45					50			

gat	gtt	gtc	att	ttg	tcg	gga	cgt	cac	ctg	gag	gga	ttg	aag	acg	gtt	307
Asp	Val	Val	Ile	Leu	Ser	Gly	Arg	His	Leu	Glu	Gly	Leu	Lys	Thr	Val	
				55			60				65					

ctt	gat	ctt	ggg	cag	tac	gac	atc	acc	atg	gtg	ggg	tca	cac	ggg	tct	355
Leu	Asp	Leu	Gly	Gln	Tyr	Asp	Ile	Thr	Met	Val	Gly	Ser	His	Gly	Ser	
				70			75			80				85		

gag	gat	tcc	tcc	cgc	ccg	cgt	acc	ctc	act	cct	gaa	gag	gta	gct	cgc	403
Glu	Asp	Ser	Ser	Arg	Pro	Arg	Thr	Leu	Thr	Pro	Glu	Glu	Val	Ala	Arg	
				90						95				100		

ctc	gcc	aag	att	gaa	gca	gat	ctg	gaa	aag	atc	gtc	gac	ggc	atc	gaa	451
Leu	Ala	Lys	Ile	Glu	Ala	Asp	Leu	Glu	Lys	Ile	Val	Asp	Gly	Ile	Glu	
				105				110					115			

ggc	gca	ttc	gtg	gag	atc	aag	cct	ttc	cac	cgc	gtg	ctg	cac	ttc	atc	499
Gly	Ala	Phe	Val	Glu	Ile	Lys	Pro	Phe	His	Arg	Val	Leu	His	Phe	Ile	
				120			125					130				

cgt	gtt	tcc	gac	aag	gac	aaa	gtc	caa	gga	atc	ctc	gcc	caa	gca	gca	547
Arg	Val	Ser	Asp	Lys	Asp	Lys	Val	Gln	Gly	Ile	Leu	Ala	Gln	Ala	Ala	
				135			140					145				

61

cac gta gac tct tcc ggc ctg aag gtt act aac ggc aag agc atc atc 595
 His Val Asp Ser Ser Gly Leu Lys Val Thr Asn Gly Lys Ser Ile Ile
 150 155 160 165

gaa tac tcc atc agc tcc acc acc aag ggc acc tgg ctg aag gaa tac 643
 Glu Tyr Ser Ile Ser Ser Thr Thr Lys Gly Thr Trp Leu Lys Glu Tyr
 170 175 180

gtt gac cgc acc gag ccc act ggt gtg att ttc ctc ggc gat gac acc 691
 Val Asp Arg Thr Glu Pro Thr Gly Val Ile Phe Leu Gly Asp Asp Thr
 185 190 195

acc gat gag cac ggt ttc aaa gct tta gaa aac gat gat cgt gcc cta 739
 Thr Asp Glu His Gly Phe Lys Ala Leu Glu Asn Asp Asp Arg Ala Leu
 200 205 210

acc gtc aag gtt ggc gaa gga gac act gca gcc aaa acc cgc gtc gac 787
 Thr Val Lys Val Gly Glu Gly Asp Thr Ala Ala Lys Thr Arg Val Asp
 215 220 225

gat gtt gat aat gtg gga att ttc cta gag aaa ctc gcc tac cac cgc 835
 Asp Val Asp Asn Val Gly Ile Phe Leu Glu Lys Leu Ala Tyr His Arg
 230 235 240 245

atg cag tat gcg gaa agc gtg cga ttg ggg att taagagagcc taaacgcacg 888
 Met Gln Tyr Ala Glu Ser Val Arg Leu Gly Ile
 250 255

aaa 891

<210> 32

<211> 256

<212> PRT

<213> Corynebacterium glutamicum

<400> 32

Met Thr Leu Thr Ile Glu Glu Ile Ala Lys Thr Lys Lys Leu Leu Val
 1 5 10 15

Val Ser Asp Phe Asp Gly Thr Ile Ala Gly Phe Ser Lys Asp Ala Tyr
 20 25 30

Asn Val Pro Ile Asn Gln Lys Ser Leu Lys Ala Val Lys Asp Leu Ser
 35 40 45

Gln Gln Ala Asp Thr Asp Val Val Ile Leu Ser Gly Arg His Leu Glu
 50 55 60

Gly Leu Lys Thr Val Leu Asp Leu Gly Gln Tyr Asp Ile Thr Met Val
 65 70 75 80

Gly Ser His Gly Ser Glu Asp Ser Ser Arg Pro Arg Thr Leu Thr Pro
 85 90 95

62

Glu	Glu	Val	Ala	Arg	Leu	Ala	Lys	Ile	Glu	Ala	Asp	Leu	Glu	Lys	Ile
			100					105					110		
Val	Asp	Gly	Ile	Glu	Gly	Ala	Phe	Val	Glu	Ile	Lys	Pro	Phe	His	Arg
		115					120					125			
Val	Leu	His	Phe	Ile	Arg	Val	Ser	Asp	Lys	Asp	Lys	Val	Gln	Gly	Ile
	130					135					140				
Leu	Ala	Gln	Ala	Ala	His	Val	Asp	Ser	Ser	Gly	Leu	Lys	Val	Thr	Asn
145					150					155					160
Gly	Lys	Ser	Ile	Ile	Glu	Tyr	Ser	Ile	Ser	Ser	Thr	Thr	Lys	Gly	Thr
			165					170						175	
Trp	Leu	Lys	Glu	Tyr	Val	Asp	Arg	Thr	Glu	Pro	Thr	Gly	Val	Ile	Phe
		180					185						190		
Leu	Gly	Asp	Asp	Thr	Thr	Asp	Glu	His	Gly	Phe	Lys	Ala	Leu	Glu	Asn
	195						200					205			
Asp	Asp	Arg	Ala	Leu	Thr	Val	Lys	Val	Gly	Glu	Gly	Asp	Thr	Ala	Ala
	210					215					220				
Lys	Thr	Arg	Val	Asp	Asp	Val	Asp	Asn	Val	Gly	Ile	Phe	Leu	Glu	Lys
225				230						235					240
Leu	Ala	Tyr	His	Arg	Met	Gln	Tyr	Ala	Glu	Ser	Val	Arg	Leu	Gly	Ile
			245						250					255	

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<210> 33
<211> 2556
<212> DNA
<213> Corynebacterium glutamicum
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<220>  
<221> CDS  
<222> (101)..(2533)  
<223> RXN01239
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<400> 33
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gtaccgcacg attttgccta acttttaagg gtgtttcatc atg gca cgt cca att 115
Met Ala Arg Pro Ile
1 5
tcc gca acg tac agg ctt caa atg cga gga cct caa gca gat agc gcc 163
Ser Ala Thr Tyr Arg Leu Gln Met Arg Gly Pro Gln Ala Asp Ser Ala
10 15 20
ggg cgt tca ttt ggt ttt gcg cag gcc aaa gcc cag ctt ccc tat ctg 211
Gly Arg Ser Phe Gly Phe Ala Gln Ala Lys Ala Gln Leu Pro Tyr Leu
25 30 35
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63

aag aag cta ggc atc agc cac ctg tac ctc tcc cct att ttt acg gcc	259
Lys Lys Leu Gly Ile Ser His Leu Tyr Leu Ser Pro Ile Phe Thr Ala	
40 45 50	
atg cca gat tcc aat cat ggc tac gat gtc att gat ccc acc acc atc	307
Met Pro Asp Ser Asn His Gly Tyr Asp Val Ile Asp Pro Thr Thr Ile	
55 60 65	
aat gaa gag ctc ggt ggc atg gag ggt ctt cga gat ctt gcc gca gct	355
Asn Glu Glu Leu Gly Gly Met Glu Gly Leu Arg Asp Leu Ala Ala Ala	
70 75 80 85	
aca cac gag ttg ggc atg ggc atc atc att gat att gtt ccc aac cat	403
Thr His Glu Leu Gly Met Gly Ile Ile Ile Asp Ile Val Pro Asn His	
90 95 100	
tta ggt gtt gcc gtt cca cat ttg aat cct tgg tgg tgg gat gtt cta	451
Leu Gly Val Ala Val Pro His Leu Asn Pro Trp Trp Trp Asp Val Leu	
105 110 115	
aaa aac ggc aaa gat tcc gct ttt gag ttc tat ttc gat att gac tgg	499
Lys Asn Gly Lys Asp Ser Ala Phe Glu Phe Tyr Phe Asp Ile Asp Trp	
120 125 130	
cac gaa gac aac ggt tct ggt ggc aag ctg ggc atg ccg att ctg ggt	547
His Glu Asp Asn Gly Ser Gly Gly Lys Leu Gly Met Pro Ile Leu Gly	
135 140 145	
gct gaa ggc gat gaa gac aag ctg gaa ttc gcg gag ctt gat gga gag	595
Ala Glu Gly Asp Glu Asp Lys Leu Glu Phe Ala Glu Leu Asp Gly Glu	
150 155 160 165	
aaa gtg ctc aaa tat ttt gac cac ctc ttc cca atc gcg cct ggt acc	643
Lys Val Leu Lys Tyr Phe Asp His Leu Phe Pro Ile Ala Pro Gly Thr	
170 175 180	
gaa gaa ggg aca ccg caa gaa gtc tac aag cgc cag cat tac cgc ctg	691
Glu Glu Gly Thr Pro Gln Glu Val Tyr Lys Arg Gln His Tyr Arg Leu	
185 190 195	
cag ttc tgg cgc gat ggc gtg atc aac ttc cgt cgc ttc ttt tcc gtg	739
Gln Phe Trp Arg Asp Gly Val Ile Asn Phe Arg Arg Phe Phe Ser Val	
200 205 210	
aat acg ttg gct ggc atc agg caa gaa gat ccc tta gtg ttt gaa cat	787
Asn Thr Leu Ala Gly Ile Arg Gln Glu Asp Pro Leu Val Phe Glu His	
215 220 225	
act cat cgt ctg ctg cgc gaa ttg gtg gcg gaa gac ctc att gac ggc	835
Thr His Arg Leu Leu Arg Glu Leu Val Ala Glu Asp Leu Ile Asp Gly	
230 235 240 245	

64

gtg cgc gtc gat cac ccc gac ggg ctt tcc gat cct ttt gga tat ctg	883
Val Arg Val Asp His Pro Asp Gly Leu Ser Asp Pro Phe Gly Tyr Leu	
250 255 260	
cac aga ctc cgc gac ctc att gga cct gac cgc tgg ctg atc atc gaa	931
His Arg Leu Arg Asp Leu Ile Gly Pro Asp Arg Trp Leu Ile Ile Glu	
265 270 275	
aag atc ttg agc gtt gat gaa cca ctc gat ccc cgc ctg gcc gtt gat	979
Lys Ile Leu Ser Val Asp Glu Pro Leu Asp Pro Arg Leu Ala Val Asp	
280 285 290	
ggc acc act ggc tac gac gcc ctc cgt gaa ctc gac ggc gtg ttt atc	1027
Gly Thr Thr Gly Tyr Asp Ala Leu Arg Glu Leu Asp Gly Val Phe Ile	
295 300 305	
tcc cga gaa tct gag gac aaa ttc tcc atg ctg gcg ctg acc cac agt	1075
Ser Arg Glu Ser Glu Asp Lys Phe Ser Met Leu Ala Leu Thr His Ser	
310 315 320 325	
gga tcc acc tgg gat gaa cgc gcc ctc aaa tcc acg gag gaa agc ctc	1123
Gly Ser Thr Trp Asp Glu Arg Ala Leu Lys Ser Thr Glu Glu Ser Leu	
330 335 340	
aaa cga gtc gtc gcc caa caa gaa ctc gca gcc gaa atc tta agg ctc	1171
Lys Arg Val Val Ala Gln Gln Glu Leu Ala Ala Glu Ile Leu Arg Leu	
345 350 355	
gcc cgc gcc atg cgc cgc gat aac ttc tcc acc gca ggc acc aac gtc	1219
Ala Arg Ala Met Arg Arg Asp Asn Phe Ser Thr Ala Gly Thr Asn Val	
360 365 370	
acc gaa gac aaa ctt agc gaa acc atc atc gaa tta gtc gcc gcc atg	1267
Thr Glu Asp Lys Leu Ser Glu Thr Ile Ile Glu Leu Val Ala Ala Met	
375 380 385	
ccc gtc tac cgc gcc gac tac atc tcc ctc tca cgc acc acc gcc acc	1315
Pro Val Tyr Arg Ala Asp Tyr Ile Ser Leu Ser Arg Thr Thr Ala Thr	
390 395 400 405	
gtc atc gcg gag atg tcc aaa cgc ttc ccc tcc cgg cgt gac gca ctc	1363
Val Ile Ala Glu Met Ser Lys Arg Phe Pro Ser Arg Arg Asp Ala Leu	
410 415 420	
gac ctc atc gcg gcc gcc cta ctt ggc aat ggc gag gcc aaa atc cgc	1411
Asp Leu Ile Ala Ala Ala Leu Leu Gly Asn Gly Glu Ala Lys Ile Arg	
425 430 435	
ttc gct caa gtc tgc ggc gcc gtc atg gct aaa ggt gtg gaa gac acc	1459
Phe Ala Gln Val Cys Gly Ala Val Met Ala Lys Gly Val Glu Asp Thr	
440 445 450	

65

acc ttc tac cgc gca tct agg ctc gtt gca ttg caa gaa gtc ggt ggc	1507
Thr Phe Tyr Arg Ala Ser Arg Leu Val Ala Leu Gln Glu Val Gly Gly	
455 460 465	
gcg ccg ggg aga ttc ggc gtc tcc gct gca gaa ttc cac ttg ctg cag	1555
Ala Pro Gly Arg Phe Gly Val Ser Ala Ala Glu Phe His Leu Leu Gln	
470 475 480 485	
gaa gaa cgc agc ctg ctg tgg cca cgc acc atg acc acc ttg tcc acg	1603
Glu Glu Arg Ser Leu Leu Trp Pro Arg Thr Met Thr Thr Leu Ser Thr	
490 495 500	
cat gac acc aaa cgt ggc gaa gat acc cgc gcc cgc atc atc tcc ctg	1651
His Asp Thr Lys Arg Gly Glu Asp Thr Arg Ala Arg Ile Ile Ser Leu	
505 510 515	
tct gaa gtc ccc gat atg tac tcc gag ctg gtc aat cgt gtt ttc gcg	1699
Ser Glu Val Pro Asp Met Tyr Ser Glu Leu Val Asn Arg Val Phe Ala	
520 525 530	
gtg ctc ccc gcg cca gac ggc gca acg ggc agt ttc ctc cta caa aac	1747
Val Leu Pro Ala Pro Asp Gly Ala Thr Gly Ser Phe Leu Leu Gln Asn	
535 540 545	
ctg ctg ggc gta tgg ccc gcc gac ggc gtg atc acc gat gcg ctg cgc	1795
Leu Leu Gly Val Trp Pro Ala Asp Gly Val Ile Thr Asp Ala Leu Arg	
550 555 560 565	
gat cga ttc agg gaa tac gcc cta aaa gct atc cgc gaa gca tcc aca	1843
Asp Arg Phe Arg Glu Tyr Ala Leu Lys Ala Ile Arg Glu Ala Ser Thr	
570 575 580	
aaa acc acg tgg gtg gac ccc aac gag tcc ttc gag gct gcg gtc tgc	1891
Lys Thr Thr Trp Val Asp Pro Asn Glu Ser Phe Glu Ala Ala Val Cys	
585 590 595	
gat tgg gtg gaa gcg ctt ttc gac gga ccc tcc acc tca cta atc acc	1939
Asp Trp Val Glu Ala Leu Phe Asp Gly Pro Ser Thr Ser Leu Ile Thr	
600 605 610	
gaa ttt gtc tcc cac atc aac cgt ggc tct gtg caa atc tcc tta ggc	1987
Glu Phe Val Ser His Ile Asn Arg Gly Ser Val Gln Ile Ser Leu Gly	
615 620 625	
agg aaa ctg ctg caa atg gtg ggc gct gga atc ccc gac act tac caa	2035
Arg Lys Leu Leu Gln Met Val Gly Ala Gly Ile Pro Asp Thr Tyr Gln	
630 635 640 645	
gga act gag ttt tta gaa gac tcc ctg gta gat ccc gat aac cga cgc	2083
Gly Thr Glu Phe Leu Glu Asp Ser Leu Val Asp Pro Asp Asn Arg Arg	
650 655 660	

66

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ttt gtt gat tac acc gcc aga gaa caa gtc ctg gag cgc ctg caa acc 2131
Phe Val Asp Tyr Thr Ala Arg Glu Gln Val Leu Glu Arg Leu Gln Thr
665 670 675

tgg gct tgg acg cag gtt aat tcg gta gaa gac ttg gtg gat aac gcc 2179
Trp Ala Trp Thr Gln Val Asn Ser Val Glu Asp Leu Val Asp Asn Ala
680 685 690

gac atc gcc aaa atg gcc gtg gtc cat aaa tcc ctc gag ttg cgt gct 2227
Asp Ile Ala Lys Met Ala Val Val His Lys Ser Leu Glu Leu Arg Ala
695 700 705

gaa ttt cgt gca agc ttt gtt ggt gga gat cat cag gca gta ttt ggc 2275
Glu Phe Arg Ala Ser Phe Val Gly Gly Asp His Gln Ala Val Phe Gly
710 715 720 725

gaa ggt cgc gca gaa tcc cac atc atg ggc atc gcc cgc ggt aca gac 2323
Glu Gly Arg Ala Glu Ser His Ile Met Gly Ile Ala Arg Gly Thr Asp
730 735 740

cga aac cac ctc aac atc att gct ctt gct acc cgt cga cca ctg atc 2371
Arg Asn His Leu Asn Ile Ile Ala Leu Ala Thr Arg Arg Pro Leu Ile
745 750 755

ttg gaa gac cgt ggc gga tgg tat gac acc acc gtc acg ctt cct ggt 2419
Leu Glu Asp Arg Gly Gly Trp Tyr Asp Thr Thr Val Thr Leu Pro Gly
760 765 770

gga caa tgg gaa gac agg ctc acc ggg caa cgc ttc agt ggt gtt gtc 2467
Gly Gln Trp Glu Asp Arg Leu Thr Gly Gln Arg Phe Ser Gly Val Val
775 780 785

cca gcc acc gat ttg ttc tca cat cta ccc gta tct ttg ttg gtt tta 2515
Pro Ala Thr Asp Leu Phe Ser His Leu Pro Val Ser Leu Leu Val Leu
790 795 800 805

gta ccc gat agt gag ttt tgatccctgc acaggaaagt tag 2556
Val Pro Asp Ser Glu Phe
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<210> 34

<211> 811

<212> PRT

<213> Corynebacterium glutamicum

<400> 34

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Gln Ala Asp Ser Ala Gly Arg Ser Phe Gly Phe Ala Gln Ala Lys Ala
20 25 30

Gln Leu Pro Tyr Leu Lys Lys Leu Gly Ile Ser His Leu Tyr Leu Ser
35 40 45

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67

Pro Ile Phe Thr Ala Met Pro Asp Ser Asn His Gly Tyr Asp Val Ile
 50 55 60

Asp Pro Thr Thr Ile Asn Glu Glu Leu Gly Gly Met Glu Gly Leu Arg
 65 70 75 80

Asp Leu Ala Ala Ala Thr His Glu Leu Gly Met Gly Ile Ile Ile Asp
 85 90 95

Ile Val Pro Asn His Leu Gly Val Ala Val Pro His Leu Asn Pro Trp
 100 105 110

Trp Trp Asp Val Leu Lys Asn Gly Lys Asp Ser Ala Phe Glu Phe Tyr
 115 120 125

Phe Asp Ile Asp Trp His Glu Asp Asn Gly Ser Gly Gly Lys Leu Gly
 130 135 140

Met Pro Ile Leu Gly Ala Glu Gly Asp Glu Asp Lys Leu Glu Phe Ala
 145 150 155 160

Glu Leu Asp Gly Glu Lys Val Leu Lys Tyr Phe Asp His Leu Phe Pro
 165 170 175

Ile Ala Pro Gly Thr Glu Glu Gly Thr Pro Gln Glu Val Tyr Lys Arg
 180 185 190

Gln His Tyr Arg Leu Gln Phe Trp Arg Asp Gly Val Ile Asn Phe Arg
 195 200 205

Arg Phe Phe Ser Val Asn Thr Leu Ala Gly Ile Arg Gln Glu Asp Pro
 210 215 220

Leu Val Phe Glu His Thr His Arg Leu Leu Arg Glu Leu Val Ala Glu
 225 230 235 240

Asp Leu Ile Asp Gly Val Arg Val Asp His Pro Asp Gly Leu Ser Asp
 245 250 255

Pro Phe Gly Tyr Leu His Arg Leu Arg Asp Leu Ile Gly Pro Asp Arg
 260 265 270

Trp Leu Ile Ile Glu Lys Ile Leu Ser Val Asp Glu Pro Leu Asp Pro
 275 280 285

Arg Leu Ala Val Asp Gly Thr Thr Gly Tyr Asp Ala Leu Arg Glu Leu
 290 295 300

Asp Gly Val Phe Ile Ser Arg Glu Ser Glu Asp Lys Phe Ser Met Leu
 305 310 315 320

Ala Leu Thr His Ser Gly Ser Thr Trp Asp Glu Arg Ala Leu Lys Ser
 325 330 335

Thr Glu Glu Ser Leu Lys Arg Val Val Ala Gln Gln Glu Leu Ala Ala
 340 345 350

68

Glu	Ile	Leu	Arg	Leu	Ala	Arg	Ala	Met	Arg	Arg	Asp	Asn	Phe	Ser	Thr
		355					360					365			
Ala	Gly	Thr	Asn	Val	Thr	Glu	Asp	Lys	Leu	Ser	Glu	Thr	Ile	Ile	Glu
		370					375					380			
Leu	Val	Ala	Ala	Met	Pro	Val	Tyr	Arg	Ala	Asp	Tyr	Ile	Ser	Leu	Ser
385				390					395			400			
Arg	Thr	Thr	Ala	Thr	Val	Ile	Ala	Glu	Met	Ser	Lys	Arg	Phe	Pro	Ser
		405					410					415			
Arg	Arg	Asp	Ala	Leu	Asp	Leu	Ile	Ala	Ala	Ala	Leu	Leu	Gly	Asn	Gly
		420					425					430			
Glu	Ala	Lys	Ile	Arg	Phe	Ala	Gln	Val	Cys	Gly	Ala	Val	Met	Ala	Lys
		435					440					445			
Gly	Val	Glu	Asp	Thr	Thr	Phe	Tyr	Arg	Ala	Ser	Arg	Leu	Val	Ala	Leu
450				455					460						
Gln	Glu	Val	Gly	Gly	Ala	Pro	Gly	Arg	Phe	Gly	Val	Ser	Ala	Ala	Glu
465				470					475			480			
Phe	His	Leu	Leu	Gln	Glu	Glu	Arg	Ser	Leu	Leu	Trp	Pro	Arg	Thr	Met
		485					490					495			
Thr	Thr	Leu	Ser	Thr	His	Asp	Thr	Lys	Arg	Gly	Glu	Asp	Thr	Arg	Ala
		500					505					510			
Arg	Ile	Ile	Ser	Leu	Ser	Glu	Val	Pro	Asp	Met	Tyr	Ser	Glu	Leu	Val
		515					520					525			
Asn	Arg	Val	Phe	Ala	Val	Leu	Pro	Ala	Pro	Asp	Gly	Ala	Thr	Gly	Ser
530				535					540						
Phe	Leu	Leu	Gln	Asn	Leu	Leu	Gly	Val	Trp	Pro	Ala	Asp	Gly	Val	Ile
545				550					555			560			
Thr	Asp	Ala	Leu	Arg	Asp	Arg	Phe	Arg	Glu	Tyr	Ala	Leu	Lys	Ala	Ile
		565					570					575			
Arg	Glu	Ala	Ser	Thr	Lys	Thr	Thr	Trp	Val	Asp	Pro	Asn	Glu	Ser	Phe
		580					585					590			
Glu	Ala	Ala	Val	Cys	Asp	Trp	Val	Glu	Ala	Leu	Phe	Asp	Gly	Pro	Ser
		595					600					605			
Thr	Ser	Leu	Ile	Thr	Glu	Phe	Val	Ser	His	Ile	Asn	Arg	Gly	Ser	Val
610				615					620						
Gln	Ile	Ser	Leu	Gly	Arg	Lys	Leu	Leu	Gln	Met	Val	Gly	Ala	Gly	Ile
625				630					635			640			
Pro	Asp	Thr	Tyr	Gln	Gly	Thr	Glu	Phe	Leu	Glu	Asp	Ser	Leu	Val	Asp
		645					650					655			

Pro	Asp	Asn	Arg	Arg	Phe	Val	Asp	Tyr	Thr	Ala	Arg	Glu	Gln	Val	Leu		
660							665					670					
Glu	Arg	Leu	Gln	Thr	Trp	Ala	Trp	Thr	Gln	Val	Asn	Ser	Val	Glu	Asp		
675							680					685					
Leu	Val	Asp	Asn	Ala	Asp	Ile	Ala	Lys	Met	Ala	Val	Val	His	Lys	Ser		
690							695					700					
Leu	Glu	Leu	Arg	Ala	Glu	Phe	Arg	Ala	Ser	Phe	Val	Gly	Gly	Asp	His		
705							710					715				720	
Gln	Ala	Val	Phe	Gly	Glu	Gly	Arg	Ala	Glu	Ser	His	Ile	Met	Gly	Ile		
725							730					735					
Ala	Arg	Gly	Thr	Asp	Arg	Asn	His	Leu	Asn	Ile	Ile	Ala	Leu	Ala	Thr		
740							745					750					
Arg	Arg	Pro	Leu	Ile	Leu	Glu	Asp	Arg	Gly	Gly	Trp	Tyr	Asp	Thr	Thr		
755							760					765					
Val	Thr	Leu	Pro	Gly	Gly	Gln	Trp	Glu	Asp	Arg	Leu	Thr	Gly	Gln	Arg		
770							775					780					
Phe	Ser	Gly	Val	Val	Pro	Ala	Thr	Asp	Leu	Phe	Ser	His	Leu	Pro	Val		
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Ser	Leu	Leu	Val	Leu	Val	Pro	Asp	Ser	Glu	Phe							
805							810										

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<210> 35
<211> 1953
<212> DNA
<213> Corynebacterium glutamicum
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<222> (101)..(1930)  
<223> RXA02645
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                                         Met Leu Lys Asp Leu
                                         1             5

acc ggc ctg agg gag ttg gta ttg cgt gag atg tgc cat agc atc tca 163
Thr Gly Leu Arg Glu Leu Val Leu Arg Glu Met Cys His Ser Ile Ser
          10             15             20

cat ctt agc tgc cca acc ggc agc att ttc act agc ctg gtg gcc atg 211
His Leu Ser Ser Pro Thr Gly Ser Ile Phe Thr Ser Leu Val Ala Met
          25             30             35

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70

ttg acc tcg caa agc ttt tca gtg tgg gct cca ctt ccc cac gat gta	259
Leu Thr Ser Gln Ser Phe Ser Val Trp Ala Pro Leu Pro His Asp Val	
40 45 50	
cat ctg atc ctc aac ggc gaa acc ctc ccc atg cac aaa acg gag ggc	307
His Leu Ile Leu Asn Gly Glu Thr Leu Pro Met His Lys Thr Glu Gly	
55 60 65	
agc tgg tgg cgc gcc gag atc gcg ccc aag gcc ggc gat cgt tac ggt	355
Ser Trp Trp Arg Ala Glu Ile Ala Pro Lys Ala Gly Asp Arg Tyr Gly	
70 75 80 85	
ttt tcg ctt ttc gac ggc tcc tcc tgg tca aaa acc ctc ccc gat ccc	403
Phe Ser Leu Phe Asp Gly Ser Ser Trp Ser Lys Thr Leu Pro Asp Pro	
90 95 100	
cgc tcc aca tct caa cca gac ggg gtt cat ggt tta agt gaa gtc tcc	451
Arg Ser Thr Ser Gln Pro Asp Gly Val His Gly Leu Ser Glu Val Ser	
105 110 115	
gat gat tcc tat ctg tgg ggt gac cag cag tgg act ggc cga att ctc	499
Asp Asp Ser Tyr Leu Trp Gly Asp Gln Gln Trp Thr Gly Arg Ile Leu	
120 125 130	
cct ggc tcg gtg tta tat gag ctg cat gtg ggc acc ttt agt gaa gat	547
Pro Gly Ser Val Leu Tyr Glu Leu His Val Gly Thr Phe Ser Glu Asp	
135 140 145	
gga acg ttt gag gga gtc gtc gac aag ctt cct tat ctg cgc gac ctc	595
Gly Thr Phe Glu Gly Val Val Asp Lys Leu Pro Tyr Leu Arg Asp Leu	
150 155 160 165	
ggc gtg acc gcc atc gaa ctt tta ccc gtg cag ccc ttt ggc ggc aac	643
Gly Val Thr Ala Ile Glu Leu Leu Pro Val Gln Pro Phe Gly Gly Asn	
170 175 180	
cgc aat tgg ggc tac gac ggg gtg ctg tgg cac gcc gtc cat gca ggc	691
Arg Asn Trp Gly Tyr Asp Gly Val Leu Trp His Ala Val His Ala Gly	
185 190 195	
tac ggc ggt ccg gcg ggc ttg aaa aag ctt atc gac gcc tcc cac cag	739
Tyr Gly Gly Pro Ala Gly Leu Lys Lys Leu Ile Asp Ala Ser His Gln	
200 205 210	
gcc ggc atc gcc gtc tac tta gac gtc gtg tac aac cac ttc ggc ccc	787
Ala Gly Ile Ala Val Tyr Leu Asp Val Val Tyr Asn His Phe Gly Pro	
215 220 225	
gac ggc aac tac aac ggg caa ttt ggc ccc tac acc tct ggc ggc agc	835
Asp Gly Asn Tyr Asn Gly Gln Phe Gly Pro Tyr Thr Ser Gly Gly Ser	
230 235 240 245	

71

acc ggc tgg ggc gac gtg gtc aac atc aac ggc cat gat tca gat gaa	883
Thr Gly Trp Gly Asp Val Val Asn Ile Asn Gly His Asp Ser Asp Glu	
250 255 260	
gtc cgc aat tat att ctc gac gcc gca cgc cag tgg ttc gaa gat ttt	931
Val Arg Asn Tyr Ile Leu Asp Ala Ala Arg Gln Trp Phe Glu Asp Phe	
265 270 275	
cac gtt gat ggg ctc cgc ctc gat gcg gtg cat tct ctc gat gat cgc	979
His Val Asp Gly Leu Arg Leu Asp Ala Val His Ser Leu Asp Asp Arg	
280 285 290	
ggc gcc tat tcc cta ctt gcg cag ctg acc atg gtg gcc gag gat gtc	1027
Gly Ala Tyr Ser Leu Leu Ala Gln Leu Thr Met Val Ala Glu Asp Val	
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tcc gca caa aca ggc atc cca cgc tca ttg att gca gaa tct gaa ctc	1075
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Asn Asp Pro Lys Phe Val Thr Ser Arg Glu Ala Gly Gly Phe Gly Leu	
330 335 340	
gat gca cag tgg gtt gac gat atc cac cac gcc ctc cat gcc ctc gtt	1171
Asp Ala Gln Trp Val Asp Asp Ile His His Ala Leu His Ala Leu Val	
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tct ggc gaa cgc aat ggt tat tac agc gat ttc gga tct gtc gac aca	1219
Ser Gly Glu Arg Asn Gly Tyr Tyr Ser Asp Phe Gly Ser Val Asp Thr	
360 365 370	
tta gcc aaa acc ctg cgt gaa gta ttt gaa cac acc gga aac tac tcc	1267
Leu Ala Lys Thr Leu Arg Glu Val Phe Glu His Thr Gly Asn Tyr Ser	
375 380 385	
acg tac cgc gga cgc aac cac ggc cgc cct gtg cac ccc gat atc acc	1315
Thr Tyr Arg Gly Arg Asn His Gly Arg Pro Val His Pro Asp Ile Thr	
390 395 400 405	
cct gcc tcg cgc ttt gtc acc tac acc acc acc cat gat cag acc ggc	1363
Pro Ala Ser Arg Phe Val Thr Tyr Thr Thr Thr His Asp Gln Thr Gly	
410 415 420	
aac cgc gca atc ggc gac cgt cct tcc acg act ctc acc ccg gaa cag	1411
Asn Arg Ala Ile Gly Asp Arg Pro Ser Thr Thr Leu Thr Pro Glu Gln	
425 430 435	
cag gtg ttg aag gca gcc att atc tac agc tcg ccg tat acc ccg atg	1459
Gln Val Leu Lys Ala Ala Ile Ile Tyr Ser Ser Pro Tyr Thr Pro Met	
440 445 450	

72

ttg ttt atg ggt gaa gaa ttc gga gcc acc acc cca ttc gcc ttc ttt 1507
 Leu Phe Met Gly Glu Glu Phe Gly Ala Thr Thr Pro Phe Ala Phe Phe
 455 460 465

tgc tcc cac acc gac ccc gag ctc aac cgg cta acc tcc gag ggc cgc 1555
 Cys Ser His Thr Asp Pro Glu Leu Asn Arg Leu Thr Ser Glu Gly Arg
 470 475 480 485

aaa cgg gaa ttc gca cgc ctt ggc tgg aac gcc gac gac atc ccc tcc 1603
 Lys Arg Glu Phe Ala Arg Leu Gly Trp Asn Ala Asp Asp Ile Pro Ser
 490 495 500

ccc gag ctg gaa tcc acc ttc acc tcc tcc aaa ctc gat tgg gag ttc 1651
 Pro Glu Leu Glu Ser Thr Phe Thr Ser Ser Lys Leu Asp Trp Glu Phe
 505 510 515

act gcg gag cag cgc cgc atc aac gac gct tac aag cag ctg ttg cac 1699
 Thr Ala Glu Gln Arg Arg Ile Asn Asp Ala Tyr Lys Gln Leu Leu His
 520 525 530

ctg cgg cac acc ttg ggc ttc tcc caa cca aac ttg ctc aca ctc gag 1747
 Leu Arg His Thr Leu Gly Phe Ser Gln Pro Asn Leu Leu Thr Leu Glu
 535 540 545

gtt gag cac ggc gag aac tgg cta tcg atg gcc aat ggt cgc ggc cga 1795
 Val Glu His Gly Glu Asn Trp Leu Ser Met Ala Asn Gly Arg Gly Arg
 550 555 560 565

att ctg gcg aat ttc tcc gac gac acc atc acc gtc ccg ctt ggc ggc 1843
 Ile Leu Ala Asn Phe Ser Asp Asp Thr Ile Thr Val Pro Leu Gly Gly
 570 575 580

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 Glu Leu Ile Tyr Ser Phe Thr Ser Pro Thr Val Thr Asp Thr Ser Thr
 585 590 595

acc ctt cag ccg tgg ggc ttt gcg atc ctg acc cga aac tagaaaaag 1940
 Thr Leu Gln Pro Trp Gly Phe Ala Ile Leu Thr Arg Asn
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ccacctcgat tga 1953

<210> 36
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 <212> PRT
 <213> *Corynebacterium glutamicum*

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 Cys His Ser Ile Ser His Leu Ser Ser Pro Thr Gly Ser Ile Phe Thr
 20 25 30

73

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 35 40 45
 Leu Pro His Asp Val His Leu Ile Leu Asn Gly Glu Thr Leu Pro Met
 50 55 60
 His Lys Thr Glu Gly Ser Trp Trp Arg Ala Glu Ile Ala Pro Lys Ala
 65 70 75 80
 Gly Asp Arg Tyr Gly Phe Ser Leu Phe Asp Gly Ser Ser Trp Ser Lys
 85 90 95
 Thr Leu Pro Asp Pro Arg Ser Thr Ser Gln Pro Asp Gly Val His Gly
 100 105 110
 Leu Ser Glu Val Ser Asp Asp Ser Tyr Leu Trp Gly Asp Gln Gln Trp
 115 120 125
 Thr Gly Arg Ile Leu Pro Gly Ser Val Leu Tyr Glu Leu His Val Gly
 130 135 140
 Thr Phe Ser Glu Asp Gly Thr Phe Glu Gly Val Val Asp Lys Leu Pro
 145 150 155 160
 Tyr Leu Arg Asp Leu Gly Val Thr Ala Ile Glu Leu Leu Pro Val Gln
 165 170 175
 Pro Phe Gly Gly Asn Arg Asn Trp Gly Tyr Asp Gly Val Leu Trp His
 180 185 190
 Ala Val His Ala Gly Tyr Gly Gly Pro Ala Gly Leu Lys Lys Leu Ile
 195 200 205
 Asp Ala Ser His Gln Ala Gly Ile Ala Val Tyr Leu Asp Val Val Tyr
 210 215 220
 Asn His Phe Gly Pro Asp Gly Asn Tyr Asn Gly Gln Phe Gly Pro Tyr
 225 230 235 240
 Thr Ser Gly Gly Ser Thr Gly Trp Gly Asp Val Val Asn Ile Asn Gly
 245 250 255
 His Asp Ser Asp Glu Val Arg Asn Tyr Ile Leu Asp Ala Ala Arg Gln
 260 265 270
 Trp Phe Glu Asp Phe His Val Asp Gly Leu Arg Leu Asp Ala Val His
 275 280 285
 Ser Leu Asp Asp Arg Gly Ala Tyr Ser Leu Leu Ala Gln Leu Thr Met
 290 295 300
 Val Ala Glu Asp Val Ser Ala Gln Thr Gly Ile Pro Arg Ser Leu Ile
 305 310 315 320
 Ala Glu Ser Glu Leu Asn Asp Pro Lys Phe Val Thr Ser Arg Glu Ala
 325 330 335

74

Gly Gly Phe Gly Leu Asp Ala Gln Trp Val Asp Asp Ile His His Ala
 340 345 350
 Leu His Ala Leu Val Ser Gly Glu Arg Asn Gly Tyr Tyr Ser Asp Phe
 355 360 365
 Gly Ser Val Asp Thr Leu Ala Lys Thr Leu Arg Glu Val Phe Glu His
 370 375 380
 Thr Gly Asn Tyr Ser Thr Tyr Arg Gly Arg Asn His Gly Arg Pro Val
 385 390 395 400
 His Pro Asp Ile Thr Pro Ala Ser Arg Phe Val Thr Tyr Thr Thr Thr
 405 410 415
 His Asp Gln Thr Gly Asn Arg Ala Ile Gly Asp Arg Pro Ser Thr Thr
 420 425 430
 Leu Thr Pro Glu Gln Gln Val Leu Lys Ala Ala Ile Ile Tyr Ser Ser
 435 440 445
 Pro Tyr Thr Pro Met Leu Phe Met Gly Glu Glu Phe Gly Ala Thr Thr
 450 455 460
 Pro Phe Ala Phe Phe Cys Ser His Thr Asp Pro Glu Leu Asn Arg Leu
 465 470 475 480
 Thr Ser Glu Gly Arg Lys Arg Glu Phe Ala Arg Leu Gly Trp Asn Ala
 485 490 495
 Asp Asp Ile Pro Ser Pro Glu Leu Glu Ser Thr Phe Thr Ser Ser Lys
 500 505 510
 Leu Asp Trp Glu Phe Thr Ala Glu Gln Arg Arg Ile Asn Asp Ala Tyr
 515 520 525
 Lys Gln Leu Leu His Leu Arg His Thr Leu Gly Phe Ser Gln Pro Asn
 530 535 540
 Leu Leu Thr Leu Glu Val Glu His Gly Glu Asn Trp Leu Ser Met Ala
 545 550 555 560
 Asn Gly Arg Gly Arg Ile Leu Ala Asn Phe Ser Asp Asp Thr Ile Thr
 565 570 575
 Val Pro Leu Gly Gly Glu Leu Ile Tyr Ser Phe Thr Ser Pro Thr Val
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 Arg Asn
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75

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<222> (101)..(832)

<223> RXN02355

<400> 37

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76

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77

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78

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 Phe Asp Lys Leu Pro Asp Asp Cys Gln Gly Gln Glu Leu Lys Val His
 105 110 115
 aac gag ctg gtt gat ctt ctg cct ggt tct ttt gaa atc tcc agg act 499
 Asn Glu Leu Val Asp Leu Leu Pro Gly Ser Phe Glu Ile Ser Arg Thr
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<212> PRT

<213> Corynebacterium glutamicum

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 Ser Asn Leu Val Ile Ser Gln Pro Ser Leu Asp Phe Gly Asn Ser Ser
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 Leu Ser Gly Phe Thr Gly Cys Val Pro Phe Thr Gly Arg Ala Glu Phe
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 Phe Gln Asn Gly Glu Gln Ser Ser Val Leu Asp Ala Asp Tyr Val Thr
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 Leu Ser Ser Leu Asp Phe Asp Lys Leu Pro Asp Asp Cys Gln Gly Gln
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 Glu Leu Lys Val His Asn Glu Leu Val Asp Leu Leu Pro Gly Ser Phe
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<222> (101)..(1567)

<223> RXS00349

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gag gtt gac cgt ttg atc cgt tcc att cag gct ggt gcg acc ccg cct 499
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 Lys Met Pro Val Asn Val Phe His Val Val Gly Lys Leu Asp Thr Asn
 100 105 110
 Phe Ser Lys Leu Ser Glu Val Asp Arg Leu Ile Arg Ser Ile Gln Ala
 115 120 125
 Gly Ala Thr Pro Pro Glu Val Ala Glu Lys Ile Leu Asp Glu Leu Glu
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 Gln Ser Pro Ala Ser Tyr Gly Phe Pro Val Ala Leu Leu Gly Trp Ala
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 Phe Leu Gly Lys Lys Gly Leu Pro Thr Phe Phe Gln Asn Val Val Gly
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 340 345 350

83

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<222> (1)..(417)

<223> RXS03183

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230 235 240 245	
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250 255 260	
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Ile Asp Tyr Ala Ala Tyr Phe Asp Gly Glu His Thr Gln Trp Ala Asn	
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1212

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<213> *Corynebacterium glutamicum*

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 100 105 110

Gly Gln Asn Pro Asp Val Glu Leu Ile Ser Lys Ile Ser Ser Cys Pro
 115 120 125

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Ala Phe Pro Thr Arg Val Val Pro Val Glu Ala Leu Ala Asp Ala Leu
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 210 215 220

Phe Lys Glu Ala Ala Ile Asp Leu Tyr Gln Ser Leu Glu Gly Glu Ala
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88

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 Ser Ile Glu Asp Gln Glu Leu Leu Asn Ala Tyr Ile Leu Asp Lys Ala
 325 330 335
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 340 345 350
 Ile Pro Leu Glu Ala Val Glu Arg Leu Leu Asp
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INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 00/13143

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C12N15/54 C12N9/10 C12N1/20 C12N15/74 C12Q1/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, PAJ, BIOSIS, CHEM ABS Data, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>DATABASE SWISS-PROT [Online] EBI; 15 July 1998 (1998-07-15) "5-Methyltetrahydrofolate-homocysteine methyltransferase (EC 2.1.1.13) of Mycobacterium tuberculosis" XP002175756 Acc. No. 033259</p> <p>---</p>	<p>3-13, 15-17, 19-25, 28-33,36</p>
X	<p>DATABASE EMBL/GENBANK/DDBJ [Online] EBI; 10 July 1997 (1997-07-10) "Mycobacterium tuberculosis H37Rv complete genome; segment 95-162" XP002175757 Acc. No. Z97559</p> <p>---</p> <p style="text-align: center;">-/--</p>	<p>3-13, 15-17, 23-25, 28-33,36</p>



Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"Z" document member of the same patent family

Date of the actual completion of the international search

24 August 2001

Date of mailing of the international search report

14.11.2001

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Mata-Vicente, M

INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 00/13143

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	FUJII, K. ET AL.: "Vitamin B12-dependent Methionine Biosynthesis and Its Metabolic Role in Corynebacterium simplex ATCC 6946, a Vitamin B12-producing and Hydrocarbon-utilizable Bacterium" AGR. BIOL. CHEM., vol. 36, no. 13, 1972, pages 2323-2334, XP001016120 page 2332, left-hand column, paragraph 2 ---	1-37
A	GROSSMANN, K. ET AL: "Rapid Cloning of metK encoding methionine adenosyltransferase from Corynebacterium glutamicum by screening a genomic library on a high density colony-array" FEMS MICROBIOLOGY LETTERS, AMSTERDAM, NL, vol. 193, no. 1, 1 December 2000 (2000-12-01), pages 99-103, XP000984551 ISSN: 0378-1097 abstract ---	1-37
A	PARK, S-D ET AL: "Isolation and Analysis of metA, a Methionine Biosynthetic Gene Encoding Homoserine Acetyltransferase in Corynebacterium glutamicum" MOLECULAR AND CELLS, KOREAN SOCIETY FOR MOLECULAR SOCIETY, KR, vol. 8, no. 3, 30 June 1998 (1998-06-30), pages 286-294, XP001002218 abstract -----	1-37

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP 00/13143

Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

As far as an "in vivo" method is concerned, claim 34 is directed to a diagnostic method practised on the human/animal body and the search has been carried out and based on the alleged effects of the compound/composition.
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-37 (all partially)

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-37 (partially)

An isolated *Corynebacterium glutamicum* nucleic acid molecule consisting of SEQ ID NO:1, fragments and variants thereof and polynucleotides capable of hybridizing with them; vectors containing said nucleic acid molecules, host cells containing the vectors and method to produce a polypeptide comprising culturing said host cells.

An isolated polypeptide comprising SEQ ID NO:2, fragments and variants thereof.

Methods for producing fine chemicals comprising the use of any of the above mentioned molecules or cells.

Method for diagnosing the presence or activity of *Corynebacterium diphtheriae* comprising detecting the presence of SEQ ID NOs:1 or 2.

2. Claims: 1-37 (partially)

Idem as in subject 1, but restricted to SEQ ID NOs:3 and 4.